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The European Journal of Endocrinology Prize Winner

The *European Journal of Endocrinology* Prize is a awarded to a candidate who has contributed significantly to the advancement of the knowledge in the field of endocrinology through publication. Further inforamtion on the prize can be found on http://www.ese-hormones.org/prizes/eje.aspx. This year's recipient is Professor Felix Beuschlein. The prize will be presented as part of the ECE 2013 opening ceremony where Prof. Beuschlein will deliver his lecture. Prof. Beuschlein has also written a review article based on this lecture that has been accepted for publication in the *European Journal of Endocrinology* and can be accessed at DOI:10.1530/EJE-13-0263.

Felix Beuschlein, Germany

Felix Beuschlein was born in 1969 and went to medical school at the University of Würzburg. For his doctoral thesis he worked on projects related to adrenal tumorigenesis. This field of expertise was further intensified during a postdoctoral fellowship under the mentorship of Gary Hammer at the University of Michigan where he utilized a number of mouse models to elucidate mechanisms of adrenal growth and steroidogenesis. Upon his return to Germany at the University Hospital in Freiburg he established his own group on adrenal stem cell research and completed his medical training. In 2006, he became a full professor as the head of Endocrine Research at the University in Munich. Prof Beuschlein has received a number of awards including the Marius-Tausk and Schöller-Junkmann award from the German Endocrine Society as well as the Merck Senior Fellow Award by the Endocrine Society. He serves as the vice-president of the German Endocrine Society, as a board member of the Research Affairs Core Committee of the Endocrine Society and the executive committee of Pressor. He is the chairman of the European Network for the Study of Adrenal Tumors (ENSAT) and coordinator of an FP7 consortium.

The *European Journal of Endocrinology* Prize Lecture

Regulation of aldosterone secretion: from physiology to disease

Felix Beuschlein, University Clinic Munich, Munich, Germany

Arterial hypertension is a major cardiovascular risk factor that affects between 10 and 40% of the population in industrialized countries. Primary aldosteronism (PA) is the most common form of secondary hypertension with an estimated prevalence of ~10% in referred patients and 4% in primary care. Despite its high prevalence until recently the underlying genetic and molecular basis of this common disease has remained largely obscure with the exception of the small subgroup of patients with familial hyperaldosteronism type I.

Over the past decade a number of insights have been achieved that rely on *in vitro* cellular systems, wild-type and genetically modified *in vivo* models as well as pre-clinical and clinical studies in well-characterized patient populations. This progress has been made possible by a number of independent technical developments including that of specific hormone assays that allow measurement in small sample volumes as well as genetic techniques that enable high throughput sequencing of a large number of samples. Furthermore, animal models have provided important insights in the physiology of aldosterone regulation that have served as a starting point for investigation of mechanisms involved in autonomous aldosterone secretion. Finally, national and international networks that have built up registries and biobanks have been instrumental to foster translational research endeavors in PA.

Recently, a number of approaches including genome wide association studies, exome sequencing and mutagenesis screens have been applied in patient cohorts and *in vivo* models of PA. Thereby, it is to be expected that in the near future further pathophysiological mechanisms that result in autonomous aldosterone secretion will be unraveled.

The Geoffrey Harris Prize Winner

Professor Clarke graduated from Massey University, New Zealand (B. Agric. Sci., 1971) followed by M. Agric. Sci. (1st Class) in reproductive physiology in 1973). He then obtained his PhD at Edinburgh University, Scotland in 1976, studying endocrinology and behaviour. He has since moved to Australia and became Senior Research Fellow of NHMRC in 1986, rising to Principal Research Fellow in 1991. He became Chairman, Department of Physiology, Monash University in 2007.
Prof. Clarke's seminal studies on the measurement of hypothalamic secretion of GnRH in sentient animals were published in 1982 and he has contributed extensively to the field of reproductive neuro endocrinology for 35 years. His laboratory currently focuses on Central regulation of reproduction by kisspeptin and gonadotropin inhibitory hormone; estrogen signalling in neuroendocrine systems; control of food intake and energy expenditure by leptin and novel regulatory factors; melanocortins and reproduction; central regulation of energy expenditure.
Prof. Clarke has published 430 research papers and has received The Woodward Prize for Excellence in Research in Neuroscience (1992), a Senior Fulbright Award (1997), the Asia and Oceania Medal of Society for Endocrinology (UK) in 2001 and the TransPacific Lecturership, Endocrine Society (USA) in 2004. Most recently (2009), he was the recipient of The Geoffrey Harris Memorial Award of the International Federation of Neuroendocrinology (2009).

The Geoffrey Harris Prize Lecture

Speakers, cross-talk and chatter in reproductive neuroendocrinology

Iain Clarke, Department of Physiology, Monash University, Victoria, Melbourne, Australia

The brain controls reproduction through the secretion of GnRH, but a series of higher brain centres control the secretion of GnRH into the hypophysial portal system. Whereas GnRH might be considered the 'speaker' for the brain in the reproductive axis, there is significant 'cross-talk and chatter' relating to the higher brain centres of control. Most prominently, kisspeptin mediates the feedback effect of sex steroids on GnRH cells, which may be effected at the level of the GnRH cell bodies or the secretory terminals in the median eminence. Gonadotropin inhibitory hormone (GnIH) has also emerged as a major negative regulator of GnRH secretion and action, acting on the GnRH neurons but also being secreted into the hypophysial portal system to act on the pituitary gonadotropes. This provides 'cross-talk' and data will be reviewed for GnIH function in mammals. The 'chatter' within this system involves input from centres within the brain that respond to altered nutritional status/metabolic condition, season and stress. Alterations in energy balance change the activity of appetite regulating peptide neurons in the hypothalamus. These neurons interact with kisspeptin cells, leading to changes in GnRH secretion. Season also involves changes in kisspeptin function as well as GnIH secretion. Stress has a negative impact on the reproductive neuroendocrine system and very recent data show that this involves upregulation of GnIH gene expression. In essence, GnRH neurons are controlled by serial and converging neuronal inputs from various brain centres. The same appears to be true for GnIH neurons, so the combined output of GnRH and GnIH dictates reproductive function.

Plenary Lectures

Nutrient-sensing pathways in ageing

PL1

Nutrient-sensing pathways and ageing

Linda Partridge[1,2]
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[2]University College London, London, UK.

Research into ageing has been transformed by the discovery of single gene mutations that extend healthy lifespan in laboratory model organisms. Furthermore, the highly conserved, nutrient-sensing, insulin/IGF/TOR signalling network has proved to play a role in ageing in organisms including yeast, invertebrates and mice, and possibly also humans. This signalling network also mediates at least some of the effects of dietary restriction, which also improves health during ageing and extends lifespan in diverse organisms including rhesus monkeys. These interventions can protect against diverse ageing-related loss of function and disease, raising the prospect of the broad-spectrum, preventative medicine for ageing-related disease.

Less well understood are the biochemical mechanisms by which reduced activity of this nutrient-sensing network can improve health during ageing, and the types of ageing-related damage that are ameliorated. For instance, toxic endo- and xenobiothics can be sources of damage. One hypothesis is that the ameliorated ability to metabolize toxic compounds leads to the life span extension. The nuclear hormone receptor DHR96 is a target gene of a key component of the signalling network; it is also involved in xenobiotic metabolism. Manipulation of hormone nuclear receptors in the fly could shed light on this.

Given the highly pleiotropic effects of this signalling network, understanding its role in ageing is crucial for identifying potential drug targets to minimise health benefits with minimal side-effects.

DOI: 10.1530/endoabs.32.PL1

NET Management

PL2

Neuroendocrine tumour management

Lisa Bodei & Giovanni Paganelli
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Neuroendocrine tumours (NETs) tend to be slow growing (although aggressive forms exist) and are often diagnosed when they have already metastasised.

Treatment of NETs is typically multidisciplinary and should be individualised according to the tumour type, burden, and symptoms. Therapeutic tools in NETs include surgery, interventional radiology and medical treatments such as somatostatin analogues, interferon, chemotherapy, new targeted drugs and peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues.

Surgery is crucial in many phases, from the eradication of the primary to the debulking of metastatic lesions, especially in a multidisciplinary algorithm. It is also used to control hormonal symptoms.

Interventional radiology techniques (TACE, RFA, and radioembolization) in NETs are frequently used, due to the common spread to the liver with hypervascular metastases.

Medical therapy is used for treating symptoms and/or reducing tumour growth. Traditional chemotherapy is not commonly applied in G1–G2 NETs, since most of them are slow growing. However, schemes based on platinum derivatives and etoposide are considered in poorly differentiated and/or rapidly progressive NETs, but the choice of the regimen is based on the site of the primary, the histopathological differentiation and proliferation index.

NETs usually over-express somatostatin receptors on their cell surface, thus enabling the therapeutic use of somatostatin analogues to reduce signs and symptoms of hormone hypersecretion, to improve quality of life and to slow the tumour growth. α-Interferon has been used in NETs, with similar therapeutic effects.

Recently, the mTOR inhibitor everolimus and the tyrosine kinase inhibitor sunitinib demonstrated an impact on survival parameters in patients with pancreatic NETs and have been introduced in clinical practice. The anti-VEGF monoclonal antibody bevacizumab demonstrated an impact on survival parameters in patient with metastatic carcinoids.

Radiolabelled somatostatin analogues have been experimented in NETs for almost two decades. Several clinical trials have indicated that PRRT with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE is an efficient tool in the management of NETs. Present knowledge and clinical experience indicate that it is possible to deliver high activities, and therefore high absorbed doses, to tumours expressing sst_2 receptors, with achievement of partial and complete objective therapeutic responses in up to 30% of patients with PFS of 33–36 months and a consistent impact on survival. Side effects, involving the kidney and the bone marrow, are mild if adequate renal protection is used.

DOI: 10.1530/endoabs.32.PL2

Changing character of thyroid cancer

PL3

Changing character of thyroid cancer

Maria Alevizaki
Athens University School of Medicine, Athens, Greece.

Thyroid cancer (DTC) is diagnosed more frequently these days due to increased awareness, wider availability of detection tools and, possibly, to true increasing incidence. The epidemiology of DTC is thus changing and more 'innocent' tumours are now being detected. The management of thyroid cancer and nodules is evolving and much progress has recently been made in the diagnosis and follow-up. Management guidelines have recently been published by scientific bodies and are continuously revised incorporating the accumulating new data.

The most important changes have been made in the management of these patients, where in the past a uniform approach was used almost regardless of risk factors for recurrence. The use of lower radioiodine doses (RAI) for remnant ablation and different strategies to increase RAI uptake have been examined in randomized clinical trials and have provided useful information that may now be used in practice. Measurement of stimulated thyroglobulin (either with endogenous or exogenous TSH) and high resolution ultrasound are valuable tools used to evaluate the success of intervention and assess the possibility of 'cure'.

Furthermore, individualisation of care is supported from data about the excellent prognosis in the majority of cases. The current, 'modern day', approach involves the reassessment of risk of recurrence at regular intervals during follow up incorporating the response to treatment. The aim of such strategy is to identify the few cases that are at higher risk and modify the intensity of re-evaluation in the others. Thus the 'optimal' management of thyroid cancer patients is currently the focus of research to assure that patients will not be over-treated or over-followed up, such that they may have fewer complications and side effects and less psychological burden.

DOI: 10.1530/endoabs.32.PL3

Fondation IPSEN 2013 Endocrine Regulations Prize

PL4

Nuclear receptor 'master' coactivators of physiology and pathology

Bert O'Malley
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Nuclear receptors control gene expression by recruiting transcriptional coactivators (or corepressors). The coactivators are 'master regulators' that coordinately activate multiple distinct transcription factors and target genes and pathways to control major physiologic processes such as reproduction, inflammation, metabolism and growth. Because of their central role as 'nodes' of regulation, coactivators are major targets in the development of numerous inherited and acquired endocrine-related pathologies such as infertility, endometriosis, disorders of carbohydrate, lipid and protein metabolism, and numerous cancers. Metabolism and growth are especially prominent pathways for coordinate regulation by coactivators such as SRC-2 and SRC-3. The pleiotropic functions of coactivators in pathways are the result of combinatorial posttranslational modifications of the proteins via enzyme cascades, in conjunction with certain biological isoforms of the proteins. In metabolic diseases and cancers, the intracellular concentrations and the PTM-directed 'activities' of the coactivator proteins are critical for 'driving' the transcription-dependent physiological outcomes, However, in the case of the cancer cell's motility or in endometriosis, it is the coactivator protein's isoforms that are the major mediators of the disease progression. Thus, as a class, the coactivator proteins provide important insights to polygenic diseases. They also may

represent new 'first-in-class' types of potential targets for therapeutic intervention.

DOI: 10.1530/endoabs.32.PL4

Preventing vascular complications of diabetes

PL5

Prevention and treatment of renal and cardiovascular disease in diabetes: new aspects

Hans-Henrik Parving
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The activity of the renin–angiotensin system (RAAS) is elevated in the circulation and in various tissues and organ tissues. The increased RAAS activity plays an important role in the haemodynamic and non-haemodynamic pathogenetic mechanisms involved in kidney and vascular (CV) disease. Previous studies have demonstrated that albuminuria is not only a marker of glomerular lesions but also a progression promoter and finally a powerful predictor of the long term beneficial effect of blood pressure lowering therapy. Furthermore albuminuria ia a harbinger of CV disease. Randomized blinded studies of patients with diabetic nephropathy have demonstrated:

- Angiotensin II receptor blockers (ARB) can prevent/delay development of microalbuminuria and diabetic nephropathy independently of its beneficial blood lowering effect in hypertensive patients with type 2 diabetes and normoalbuminuria/microalbuminuria (BENEDICT and IRMA 2)
- Early ARB treatment is projected to improve life expentancy and reduce cost in hypertensive patients with type 2 diabetes and microalbuminuria. Later use of ARB in overt nephropathy is also superior to standard care, but ARB should be started earlier and continued long term.
- Two landmark studies (RENAAL and IDNT) lead to the following conclusion: 'Losartan and Irbesartan conferred significant renal benefit in patients with type 2 diabetes and nephropathy. This protection is independent of the reduction in blood pressure it causes. The ARB is genrally safe and well-tolerated'.
- Albuminuria is an important factor predicting cardiovascular risk in patients with type 2 diabetic nephropathy. Reducing albuminuria with ARB in the first 6 months appears to afford cardiovascular protection in these patients.
- The antiproteinuric effect of ARB explains a large part of the specific renoprotective characteristic. Proteinuria should be considered a risk marker for progressive renal function loss in type 2 diabetes with nephropathy, as well as target for therapy. Lowering of residual proteinuria is a goal for the future.
- Recent studies suggest that remission of nephrotic range albuminuria induced by aggressive antihypertensive treatment is associated with a slower progression in diabetic nephropathy and a substantial improved survival.
- Recent studies have suggested new renoprotective treatment modalities, e.g. ultrahigh dose of RAAS-blockade, combination of ACEI and ARB, aldosterone blockers and direct rennin inhibition.

Intensified multifactorial intervention – with tight glucose regulation and the use of renin–angiotensin system blockers, aspirin, and lipid-lowering agents – has been shown to reduce the risk of nonfatal cardiovascular disease among patients with type 2 diabetes melleitus and microalbuminuria. We evaluated whether this approach would have an effect on the rates of death from any cause and from cardiovascular causes.

In the Steno-2 study we randomly assigned 160 patients with type 2 diabetes and persistent microalbuminuria to receive either intensive therapy or conventional therapy; the mean treatment period was 7.8 years. Patients were subsequently followed observationally for a mean of 5.5 years until December 31, 2006. The primary end point at 13.3 years of follow-up was the time of death from any cause. Twenty-four patients in the intensive-therapy group died, as compared with 40 in the conventional-therapy group (hazard ratio, 0.54; 95% CI 0.32 to 0.89; $P=0.02$). Intensive therapy was associated with lower risk of death from cardiovascular causes (hazard ratio, 0.43; 95% CI, 0.19 to 0.94; $P=0.04$) and of cardiovascular events (hazard ratio, 0.41; 95% CI, 0.25 to 0.67; $P<0.001$). One patient in the intensive-therapy group had progression to end-stage renal disease, as compared with six patients in the conventional-therapy group ($P=0.04$). Fewer patients in the intensive-therapy group required retinal photocoagulation (relative risk, 0.45; 95% CI, 0.23 to 0.86; $P=0.02$). Few major side effects were reported. In at-risk patients with type 2 diabetes, intensive intervention with multiple drug combinations and behaviour modification has sustained beneficial effects with respect to vascular complications and on rates of death from any cause and from cardiovascular causes.

DOI: 10.1530/endoabs.32.PL5

The Ubiquitin System

PL6

Abstract unavailable.

DOI: 10.1530/endoabs.32.PL6

Aldosterone, Mineralocorticoid Receptors and Cardiovascular Risk: What's New?

PL7

Aldosterone, mineralocorticoid receptors and cardiovascular risk: what's new?

J Funder
Prince Henry's Institute, Monash Medical Centre, Clayton, Victoria, Australia.

Classically aldosterone acted uniquely on epithelia, and primary aldosteronism (PA) was considered uncommon and relatively benign, all of which we now know to be not the case. In 2013, we should note that:

i). Mineralocorticoid receptors (MR) evolved millions of years before aldosterone.
ii). Cortisol occupies 90–99% of all MR in the human body.
iii). MR are promiscuous, binding cortisol, aldosterone and progesterone with the same high affinity.
iv). Cortisol is bivalent: normally an antagonist of aldosterone in MR, it acts as an agonist under conditions of tissue damage/reactive oxygen species generation/redox change.
v). Cortisol is thus the cardiac/vascular MR agonist in essential hypertension (EH) and congestive heart failure (CHF).
vi). Spironolactone and eplerenone do not act primarily by denying agonist access to MR, but as inverse agonists in their own right.
vii). Around 40% of PA due to an aldosterone producing adenoma is due to somatic mutation of KCNJ5, with further somatic mutations in press.
viii). Three germline mutations causing PA have been distinguished; Familial Hyperaldosteronism Type 1 and 3 are rare (<1%), but the prevalence of FH2 is likely to be much higher.
ix). The currently accepted prevalence of PA may be too low, and the upper limit of 'normal' aldosterone too high, with evidence accruing that many patients with resistant hypertension and low renin hypertension have autonomous aldosterone secretion, i.e. PA.
x). Even at currently accepted levels for PA fewer than I% of patients are ever screened, diagnosed and specifically treated.
xi). No jurisdiction has the resources – financial or medical – to meaningfully raise this percentage.
xii). Low dose MR antagonists are safe and efficacious in EH, selectively active in resistant hypertension, and game-changing in PA; and so
xiii). Given the much higher cardiovascular risk profile of PA than in age-, sex- and blood pressure-matched EH, and that 99+% of PA remains occult, low dose MR antagonists should be included in first-line therapy for all hypertensives.

DOI: 10.1530/endoabs.32.PL7

New genes and functions in reproduction

PL8

New genes and functions in reproduction

Martin M Matzuk
Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas, USA.

For almost three decades, my group and I have been studying the functions of known proteins and deciphering the roles of novel genes and small RNAs expressed within the reproductive axis. Many of these studies have focused on the glycoprotein hormones as well as members of the transforming growth factor β superfamily. We have also used multiple strategies to identify unique germ-cell

expressed genes and probed their essential roles in knockout mouse models. These studies have led us to an understanding of the intricate pathways required for development and function of the reproductive axis in mammals.

DOI: 10.1530/endoabs.32.PL8

Human Brown Fat is on Fire

PL9

Human brown fat is on fire

Barbara Cannon
Wenner-Gren Institute, Stockholm, Sweden.

Brown adipose tissue was classically a tissue with scientifically interesting bioenergetic features, manifest by the unique presence of uncoupling protein-1 in only this tissue – but it was considered to have no metabolic significance for adult humans. The acceptance within the last years of its presence in adult humans has intensified interest in its potential ability not only to keep us warm but particularly to burn excess energy, i.e. to keep us slim, and – through its utilization of lipids and glucose – to counteract the metabolic syndrome. The analysis of its function and significance in experimental animals can now likely be extrapolated to humans. The principal conclusion is that brown adipose tissue is the sole organ responsible for classical nonshivering thermogenesis as well as for diet-induced thermogenesis, and that no other mechanisms for adaptive thermogenesis exists. The extrapolation is evidently that its activity potentially could be exploited to promote human health.

DOI: 10.1530/endoabs.32.PL9

Symposia

Metabolic surgery

S1.1

New insights in obesity pathophysiology of metabolic surgery

Gema Frühbeck[1,2]
[1]Department of Endocrinology and Nutrition, Clínica Univ. de Navarra, University of Navarra, Pamplona, Spain; [2]CIBERobn, Instituto de Salud Carlos III, Pamplona, Spain.

Obesity represents an important risk factor for type 2 diabetes mellitus (T2DM) development. The outcomes of both bariatric and metabolic surgery have provided new insights into the mechanisms responsible for body weight control and glucose homeostasis. However, at the same time the marked effects of metabolic surgery on T2DM highlights the limitations of our current knowledge underscoring the need to continue addressing relevant questions as regards the underlying mechanisms and their precise contribution in each case. As expected, weight loss improves the major factors involved in the pathogenesis of T2DM, namely insulin action and β cell function. Interestingly, over the past decades bariatric surgical procedures that divert nutrients away from the upper gastrointestinal system are more successful in producing weight loss and remission of T2DM than those that simply restrict stomach capacity. Moreover, the almost immediate beneficial effects on glucose homeostasis following bariatric surgery when no substancial change in body weight has taken place points to the existence of relevant weight loss-independent factors. This statement is based primarily on the following findings: i) the early postoperative effects of especially some types of bariatric surgery on glycemic control; ii) the long-term efficacy of different surgical procedures on T2DM resolution; iii) the effect of the duodenal-jejunal bypass (DJB), which greatly influences glucose homeostasis despite minimal weight loss; and iv) the rapid and specific hormonal response to glucose or mixed meal ingestion. Circulating insulin levels represent a summation of events that involve both β cell and non-β cell metabolic pathways. Furthermore, BMI and total body weight do not reflect differences in body composition and adiposity distribution, thereby ignoring the relationship between the different compartments (fat, muscle, liver, etc.) and metabolic risk. More research is needed to make definitive conclusions on the precise and probably multifactorial causes backing metabolic surgery effectiveness.

DOI: 10.1530/endoabs.32.S1.1

S1.2

Abstract unavailable.

DOI: 10.1530/endoabs.32.S1.2

S1.3

Abstract unavailable.

DOI: 10.1530/endoabs.32.S1.3

Cushing's Disease with negative pituitary imaging

S2.1

Abstract unavailable.

DOI: 10.1530/endoabs.32.S2.1

S2.2

Surgical approach to corticotroph adenomas poorly visible at preoperative imaging

Michael Buchfelder
Department of Neurosurgery, University of Erlangen-Nürnberg, Erlangen, Germany.

Most pituitary operations for Cushings's disease are technically difficult procedures. Since the souce of excessive ACTH-secretion in Cushing's disesase is almost invariably a pituitary microadenoma, selective resection of this minute tumor is considered the primary standard therapy. However, almost half of the tumors escape direct preoperative detection even by sophisticated magnetic resonance imaging. Thus, in these the indication for surgery is based on laboratory findings of hormone determinations, including dynamic tests and selective blood sampling from the inferior petrosal sinus. Systematic sectioning of the gland is required to find the tiny tumor. Technical supports, like intraoperative sonography, have been introduced. Following successful surgery, ACTH and cortisol levels, respectively, decrease rapidly to ideally reach subnormal levels. Such an isolated adrenocortical insufficiency is a favourable prognostic indicator for long-lasting remission and requires corticosteroid substitution. Some 65–90% of patients harbouring pituitary microadenomas experience a remission after transsphenoidal pituitary surgery in experienced centers. In most series, negative imaging is an unfavourable prognostic factor. Moreover, there is a recurrence rate of some 10–20% at 10 years. Also the technical performance of such transsphenoidal sella explorations is challenging. The sella is frequently normal sized, incompletely pneumatized and thus, more venous bleeding occurs intraoperatively than with larger adenomas. Many of the patients harbour significant comorbitities. However, in some 90% of patients microadenomas are detected intra-operatively. Not in all of these, representative tissue arrives in the pathologist's laboratory. For ill-defined tumors, lateral hemihypophysectomy, central core partial hypophysectomy or total hypophysectomy may be considered. However, the success rate of any kind of hypophysectomy in terms of normal ACTH- and cortisol secretion is lower than that of selective operations in patients with distinct radiological findings.

DOI: 10.1530/endoabs.32.S2.2

S2.3

Medical approach to Cushing's disease: results of a French multicentre study

Frederic Castinetti
Department of Endocrinology and Reference Center for Rare Pituitary Diseases, La Timone Hospital, Aix Marseille University, Marseille, France.

Surgery remains the first line treatment of Cushing's disease. However, recent studies based on long term follow-up showed that the risk of late recurrence was close to 20–30% cases, whatever the immediate post-surgical cortisol level. In other cases, surgery is not possible due to contra-indications, or refusal by the patient. Medical treatment is thus of major importance in the management of Cushing's disease, also taking into account the delay to obtain maximal efficacy of radiation techniques, when they are indicated.
This talk will be aimed at showing the main merits and pitfalls of anticortisolic drugs, first by evaluating the recently used new pituitary targeted drugs, and then by comparing them to the classical adrenal targeted anticortisolic drugs. Among these, ketoconazole should be considered as a valuable treatment. Despite a relatively low number of cases reported to date, this drug has been shown to be effective and relatively safe. We will present here the results of a French multicentric retrospective study based on more than 150 patients treated by ketoconazole for Cushing's disease, and will try to define its role in the therapeutic algorithm of this disease.

DOI: 10.1530/endoabs.32.S2.3

Female reproduction

S3.1

The transcription factor FOXL2 in ovarian physiology and pathology

Reiner Veitia
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The gene FOXL2 encodes a forkhead transcription factor whose mutations or misregulation are responsible for the blepharophimosis-ptosis-epicanthus

inversus (BPES) syndrome and more recently have been associated with ovarian granulosa cell tumors (OGCT). BPES is a genetic disorder involving craniofacial abnormalities associated with premature ovarian failure. OGCTs are endocrine malignancies, accounting for up to 5% of ovarian cancers, the treatment of which is challenging. I will summarize recent data on FOXL2 transcriptional targets and molecular partners, its post-translational modifications, its mutations and its involvement in OGCTs. In the ovarian context, FOXL2 is involved in the regulation of cholesterol and steroid metabolism, apoptosis, reactive oxygen species detoxification and cell proliferation. In addition, one of the main roles of FOXL2 is to preserve the identity of ovarian granulosa cells even at the adult stage and to prevent their transdifferentiation into Sertoli-like cells. These recent advances indicate that FOXL2 is a key factor for ovarian development and maintenance. The elucidation of the impact of FOXL2 germline and somatic mutations will ensure a better understanding of the pathogenesis of BPES and of OGCTs.

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S3.2

NR5A1/SF-1 and gonadal development and function

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Steroidogenic factor 1 (SF-1/NR5A1) is a regulator of steroid hormone biosynthesis and sex development. It was first identified in a 46,XY newborn with complete sex reversal and primary adrenal insufficiency. Most 46,XY patients carrying heterozygote NR5A1 mutations manifest with a disorder of sexual development (DSD) phenotype, although an adrenal only phenotype has also been described in one 46,XX patient. Mutations in NR5A1 may be found in up to 9% of 46,XY subjects with mild to severe DSD. Testes histology is characteristic for fat accumulation and degeneration over time similar to findings observed in patients with lipoid congenital adrenal hyperplasia (due to *StAR* mutations). Female relatives with heterozygote NR5A1 mutations show primary ovarian insufficiency (POI) at puberty. Ovarian biopsy in one reported female showed extensive fibrosis with no evidence of follicles. However, in 46,XX subjects with sporadic, non-syndromic POI, heterozygote NR5A1 mutations are only found in 1.4–8%. DSD due to heterozygote NR5A1 mutations has also been reported in offsprings of parents who were fertile but were carriers of same NR5A1 mutations. Overall, patient studies show that NR5A1 mutations present with a very broad phenotype, especially in 46,XY. Testis and ovary can be affected to different degrees. Recent findings from mice models establish a role for SF-1 in early gonadal development (sex determination) and gonadal (both testis and ovary) differentiation. In addition, SF-1 is an important transcription factor for steroid biosynthesis. However, the broad phenotypic variability associated with NR5A1 mutations remains a conundrum. Internal (e.g. mutations in other genes) as well as external (e.g. environmental) modulators may be considered but have not been found yet.

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S3.3

Progestin action in the brain

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The brain is an important target of progesterone. Already earlier studies consistently reported the presence of progesterone receptors (PR) throughout the brain. The wide distribution of brain PR has subsequently been confirmed by mRNA analysis, immunohistochemistry and more recently by immunoelectron microscopy. Surprisingly, the significance of the wide distribution of PR in the brain has largely remained unexplored, and only hypothalamic receptors have been extensively studied for their role in the regulation of female reproductive behavior. This lack of information about the brain functions of PR is unexpected, as progesterone is known to exert multiple effects on neural cells. Moreover, the neuroprotective effects of progesterone have recently received much attention. A reason why so little interest has been devoted to the brain functions of PR may be the widely accepted assumption that non-reproductive functions of progesterone may be mainly mediated by its metabolite allopregnanolone, which does not bind to PR, but to membrane γ-aminobutyric acid type A ($GABA_A$) receptors, a major inhibitory neurotransmitter receptor. It is indeed widely acknowledged that allopregnanolone has anxiolytic, anesthetic, antidepressant and anticonvulsant actions by modulating $GABA_A$ receptors. Our recent experimental studies demonstrate a key role for PR in neuronal survival after stroke, induced by the transient occlusion of the middle cerebral artery (MCAO) in mice. Importantly, we show that PR deficiency, and even haploinsufficiency, markedly increases the vulnerability of the brain to ischemic injury, resulting in increased infarct volume and poor functional outcomes. Identification of PR as a drug target for neuroprotection opens new therapeutic indications for selective synthetic progestins, already validated for contraception or hormone therapy. Thus, the neuroprotective effect of progesterone therapy could be mimicked by administration of a low dose of the potent 19-norprogesterone derivative Nestorone.

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New advances in GPCRs in endocrinology

S4.1

Abstract unavailable.

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S4.2

Biased agonism of the AT1 receptor: perspectives in drug discovery?

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The angiotensin II type 1 receptor (AT_1R) belongs to the family of seven transmembrane (7TM) receptors, also referred to as G-protein coupled receptors. The AT_1R is the primary effector of the renin–angiotensin system, and serves as a key regulator of cardiovascular physiology. The importance of the receptor is clearly illustrated be the frequent use of AT_1R blockers and ACE inhibitors in cardiovascular medicine. Upon binding of Ang II the AT_1R is signals through both G protein-dependent and -independent pathways. The G protein-dependent pathways are well established and studied in detail whereas we know less about G protein-independent pathways. Pharmacological targeted activation and blocking of the signaling cascades provides novel tools to increase the understanding of how these receptors exert their cellular functions and importantly it present a new clinical potential. This so-called biased agonism (or functional selectivity) has been studied extensively for the last decade and the focus is still increasing. In this talk I will introduce the concept of AT_1R mediated biased agonism, discuss the underlying complexity of the AT_1R signaling transduction networks and gene regulation and present the clinical potential of AT_1R biased agonists.

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S4.3

G-protein-coupled receptor heteromers as new targets for drug development

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Together with Profs Lefkowitz and Kobilka, G-protein-coupled receptors (GPCRs) have taken the stage in the 2012 Nobel award for Chemistry. The Royal Swedish Academy of Sciences states that 'About half of all medications achieve their effect through G-protein-coupled receptors'. As shown in previous talks in this session, GPCRs are not acting individually; often they form homodimers and, more interestingly, they may occur as heterodimers. Heteromers open a completely new scenario for structural and functional diversity and for drug discovery. By consensus in the field a GPCR heteromer is a macromolecular complex, composed of at least two different receptor units with biochemical properties that are demonstrably different from those of the individual components[1]. In fact a precise quaternary structure of the whole complex is needed for heteromer-specific signaling and function[4].
The seminal work by Rivero-Müller *et al.*[5] shows that transgenic mice coexpressing binding-deficient and signaling-deficient forms of LH receptor LHR can reestablish normal LH actions through intermolecular functional complementation of the mutant receptors in the absence of functional wild-type

receptors. These results provide compelling *in vivo* evidence for the physiological relevance of intermolecular cooperation in GPCR signaling. Other examples are constituted by chemokine receptors that regulate neuroendocrine actions (reviewed in Guyon & Nahon[3]) and by pineal gland adrenergic and dopamine receptors whose circadian-related heteromerization modulate melatonin synthesis and release[2].

On the one hand, the talk will provide examples of heteromer alteration in disease and the notion that current GPCR-based therapies are in fact targeting receptor heteromers. On the other hand, a variety of current strategies to design and screen heteromer-selective drugs will be presented. Advantages of heteromer-selective drugs are a more directed targeting, for instance pre- vs post-synaptic in the nervous system, and less side effects.

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A guide through the labyrinth of neuroendocrine tumours

S5.1

Tumor biology and classification of NET

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Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) constitute a heterogeneous group of neoplasms. In the last few decades, due to a substantial rise in incidence and prevalence, GEP-NETs have been included among the most common tumors of the gastrointestinal tract. Diagnosis could be challenging and a significant number of patients present with metastatic or unresecable disease. The development of appropriate tools for standardized prognostic stratification and the introduction of effective target therapies have opened new horizons for planning tailored surgical or medical management and follow-up programs for these complex neoplasms. An overview on the GEP-NETs' diagnostic and prognostic criteria proposed by the recently published WHO classification and ENETS and UICC TNM staging systems is presented, focusing on their impact on the clinical and therapeutical approaches.

The genetic events underlying the tumorigenesis of this complex group of neoplams still remain to be defined. Very recently, extensive investigations have focused on the neuroendocrine tumors of pancreatic origin (PEN). Indeed, massive-scale sequencing PEN has led to the identification of alterations of genes involved in the chromatin remodelling (*MEN1*, *DAXX*, and *ATRX)* in up to 60% of cases. Moreover, a small subgroup of patients harbored alterations in genes (PTEN and TSC2) that negatively regulate mTOR activation, for which targeted therapy in PEN already exist. Beyond alterations in protein coding genes, survey of chromosomal status have demonstrated that a high degree of genomic instability correlates with the aggressiveness of this neoplasm. Gene silencing by promoter methylation has been advocated, but a formal demonstration of the involvement of specific genes is still lacking. Expression profiling studies are furnishing valuable lists of mRNAs and noncoding RNAs that may advance further the research to discover novel markers and/or therapeutic targets.

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S5.2

Nuclear medicine imaging of neuroendocrine tumours

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Computed tomography (CT) is the basic method for morphological imaging of neuroendocrine tumours (NETs) for visualization of local tumour extent and staging of regional and distant metastases. Magnetic resonance imaging (MRI) and intravenously contrast-enhanced ultrasound (CEUS) are complementary methods but are less frequently available and are therefore predominately used when CT does not suffice. This, however, varies depending on the local situation and expertise. Endoscopic US (EUS) is superior for detection and to evaluate the local extent of gastric, duodenal and pancreatic NETs but has similarly limited availability.

Scintigraphy by ^{111}In-labelled octreotide (OctreoScan) remains the mainstay for evaluation of the NET's somatostatin receptor status and generally facilitates tumour staging and is important to assess the patient's eligibility for treatment with somatostatin analogues. Recently, various 68Ga-labelled somatostatin analogues have been tested for NET imaging by positron emission tomography (PET) in combination with CT (PET/CT). Generally, 68Ga-labelled octreotide (68Ga-DOTA-TOC, 68Ga-DOTA-NOC) and octreotate (68Ga-DOTA-TATE) are used as PET tracers. Somatostatin receptor imaging by PET has in several comparative studies performed better than scintigraphy. Centres where PET/CT with 68Ga-labelled somatostatin analogues is available are still few but the technique is fairly rapidly increasing and scintigraphy with OctreoScan has in these centres generally been abandoned. Because of existing protocols, scintigraphy with OctreoScan is still performed for patient selection when peptide receptor radio therapy (PRRT) with 177Lu-DOTA-TATE is considered. Because of the 68Ga-labelled somatostatin analogue preparations favourable pharmacokinetics, PET imaging can be performed already 30–60 min after tracer injection as compared to scintigraphy, which is generally performed at 4 and 24 h. Also the spatial resolution of PET and the image contrast is better.

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S5.3

Abstract unavailable.

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What's new in type 2 diabetes?

S6.1

The impact of our genomes on metabolic health

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For the past two decades, genetics has been widely explored as a tool for unravelling the pathogenesis of cardio-metabolic disorders. Many risk alleles for type 2 diabetes and hyperglycaemia have been detected in recent years through massive genome-wide association studies and evidence exists that most of these variants influence pancreatic β-cell function. Investigations of more detailed physiological phenotypes, are now emerging and give indications of more specific pathological mechanism for diabetes-related risk variants. Such studies have shed light on the function of some loci but also underlined the complex nature of disease mechanism. In the future, sequencing-based discovery of low-frequency variants with higher impact on intermediate diabetes-related traits is a likely scenario and identification of new pathways involved in type 2 diabetes predisposition will offer opportunities for the development of novel therapeutic and preventative approaches. Furthermore, we recently described the Illumina-based metagenomic sequencing assembly and characterisation of 3.3 million non-redundant microbial genes from faecal samples of 124 European individuals. The extensive gene catalogue has enabled us to perform studies of association of the microbial genes with human metabolic phenotypes.

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S6.2

Insulin resistance and adipose tissue

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Inability to store excess lipids in the subcutaneous adipose tissue leads to ectopic lipid deposition in several sites including the liver, skeletal muscles, intra-abdominal and pericardial depots. This, in turn, has negative consequences for degree of insulin sensitivity and the dysmetabolic state associated with insulin resistance and type 2 diabetes.
Storage of fat in the adipose tissue can either lead to inappropriate cellular enlargement (hypertrophic obesity) or the recruitment of new adipose cells (hyperplastic obesity). The latter is more favourable from a metabolic point of view and is associated with the 'obese but metabolically normal' phenotype.
The reason for this difference in storing excess lipids is related to an ability or not to recruit and differentiate new adipose cells from precursor cells. We have in a series of papers shown that hypertrophic obesity is associated with insulin resistant adipose tissue with reduced expression of IRS1, GLUT4 and several other PPARγ-regulated genes and occurred around four times more frequently in individuals with a genetic predisposition for type 2 diabetes (first degree relatives, FDR) than in individuals lacking a genetic predisposition. In recent studies, we have found that FDR exhibit an obese metabolic phenotype with inappropriately enlarged adipose cells, reduced insulin sensitivity and a dysregulated adipose tissue even in the absence of obesity while this phenotype is not found in subjects with heredity for obesity/overweight. These findings provide a link between diabetes heredity and increased sensitivity to the environment and caloric excess. Furthermore, we have recently identified a novel mechanism whereby mesenchymal stem cells and other precursor cells become committed to the adipose lineage in response to BMP4 and subsequently can undergo differentiation.

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S6.3

Mechanisms of β cell failure in type 2 diabetes

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Pancreatic β cell dysfunction and death are central in the pathogenesis of type 2 diabetes but the underlying mechanisms are not well understood. Genetic factors predispose to type 2 diabetes but, despite very large scale genome-wide association studies, the heritability of the disease remains largely unexplained. Environmental and lifestyle factors contribute to the pathogenesis of type 2 diabetes and likely explain its rapidly increasing prevalence. Elevated levels of saturated free fatty acids may cause β cell failure in insulin resistant individuals, and our work aims to clarify the molecular mechanisms involved. Epigenetic changes are an additional link for translating environmental exposures into heritable disease mechanisms. Human β cells are long-lived, and have a lifetime to acquire epigenetic alterations.
We have performed the first comprehensive DNA methylation profiling in human islets from type 2 diabetic and non-diabetic donors, identifying differential DNA methylation in genes in pathways affecting β cell function and survival. In parallel, we are mapping the human islet transcriptome by RNA-sequencing, under control condition or following exposure to the saturated fatty acid palmitate. Palmitate modified transcripts related to the endoplasmic reticulum stress response, ubiquitin and proteasome function, autophagy and apoptosis. Several transcription factors controlling β cell phenotype were inhibited by palmitate. In addition, palmitate caused a shift in alternative splicing, pointing to novel mechanisms of palmitate-induced β cell dysfunction and death.
These omic approaches will offer new insights into the pathogenesis of diabetes. A better understanding of the epigenetic dysregulation in type 2 diabetic islets and the mapping of the human islet transcriptome will advance our understanding of disease etiology.

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Translational aspects from comparative to clinical endocrinology

S7.1

Brain aromatase and endocrine disruptors in zebrafish: from basic to applied research

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Aromatase is the only enzyme converting androgens into estrogens, which are key actors in reproductive biology. Teleost fishes have two copies of the cyp19a1 gene, which encode two isoforms of aromatase: cyp19a1a encodes ovarian aromatase, while the cyp19a1b gene encodes brain aromatase. Our recent work showed that, in zebrafish, aromatase B is strongly expressed in a unique brain cell type, the radial glial cells (RGC). In mammals, such cells act as stem cells during embryonic development before disappearing in adults. On the contrary, in fishes, RGCs persist in adult where they act as neuronal progenitors allowing the brain to constantly keep growing. We have also shown that, intriguingly, the *cyp19a1b* gene is very sensitive to estrogens, through a mechanism that involves a well conserved ERE. This feature makes this gene an outstanding biomarker of xeno-estrogen exposure, and we have developed an *in vivo* assay allowing detection of estrogenic activity with a very high sensitivity. This assay is based on a transgenic zebrafish tg(*cyp19a1b*-GFP) line that expresses GFP in RGCs. By quantifying GFP expression in live fish, we show that short-term exposure of tg(*cyp19a1b*-GFP) embryos from 0 to 120 hpf to a variety of well established estrogenic compounds (estradiol, estriol, estrone, ethinylestradiol, zearalenone and its metabolites, nonyl, octyl and tert-pentylphenol, BPA, benzophenones derivates, etc.) turns on GFP expression in a concentration-dependent manner. Overall, we demonstrate the remarkable usefulness of the tg(*cyp19a1b*-GFP) embryos as a reliable, sensitive and rapid *in vivo* estrogenic screening assay. This assay nicely complements the *in vitro* assay that we have previously developed using the same promoter coupled to luciferase. As we have also evidenced an effect of estrogens on the neurogenic activity of zebrafish, abnormal exposure of fish embryos to estrogenic endocrine disruptors is likely to affect the neurogenic process.

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S7.2

Abstract unavailable.

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S7.3

Peptide hormones and receptors: why so many?

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Hormones and hormone receptors in mammals, including humans, display bewildering complexity. The evolution of peptide hormone families with multiple members has been difficult to resolve because of their short sequences. Their G-protein-coupled receptor families are challenging because they display variable sequence rates along the proteins, between species, and over time. My laboratory has therefore combined analyses of sequences with investigation of conserved synteny, i.e. comparisons of the chromosomal locations of the genes between species. This facilitates identification of true species homologs. Using these approaches we have been able to disentangle the evolutionary histories of endocrine peptide and receptor families. Invariably, we find that the ancestor of vertebrates must have had more gene family members than humans have today. This ancestral complexity is due to two genome doublings in early vertebrate evolution, leading to quadruplication of the ancestral chromosome set, thereby generating additional gene copies for many peptides and receptors. The NPY-PYY-pancreatic polypeptide family once had seven receptors, only four of which still exist in humans. Today humans have three vasopressin receptors, whereas the ancestral vertebrate had six. An ancient duplicate of prolactin is present in birds and fish, but was lost in the mammalian lineage. The somatostatin receptor family previously had six members, one of which was lost before the origin of mammals. One of the most extreme cases is the receptor family for the somatostatin-related peptide urotensin II: mammals have only a single receptor, but our ancestors possessed no less then five, all of which still exist in some vertebrates such as a lizard and a turtle. Thus, although our neuroendocrine system is certainly quite complex, we have clearly degenerated by gene loss. Furthermore, many fishes have gained additional gene copies by another genome doubling as well as local gene duplications. An important question arises: which functions have we and other mammals lost that still exist in other vertebrates?

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Action of glucocorticoids on bone

S8.1

Glucocorticoid action on the skeleton

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Glucorticoids (GCs) belong since their discovery to the standard therapy of rheumatoid arthritis (RA), a severe auto-inflammatory bone disease. One major side effect of GCs affects the skeleton itself leading to GC induced osteoporosis (GIO), the most secondary osteoporosis.
To optimize anti-inflammatory therapies utilizing steroids a profound understanding of GC action on the interface of inflammation and skeletal integrity is required.
GCs act via a receptor (GR) that can alter gene expression by binding as a dimer to GC responsible elements in the promoter region of target genes or by interacting with and thus interfering with other transcription factors as a monomer. We determined the contribution of molecular mechanisms and cell types critically involved in anti-inflammatory effects of GCs in RA and on skeleton using conditional and function selective GR knockout mice.
We made the surprising observation that for suppression of inflammation in several arthritis models GR dimerization is absolutely required in part including a specific action in T cells.
In contrast for GC induced bone loss we demonstrated in a model of GIO that unexpectedly interaction of the GR monomer with AP-1, but not NF-kB in osteoblasts is decisive for bone loss.
Our findings define new criteria for SEGRM that act anti-inflammatory and protect the bone. Indeed we identified one lead-compound that still suppresses NF-kB dependent gene expression but does not affect osteoblast differentiation and activity.
Furthermore we identified novel GR target genes by functional genomics and developed a screening platform for novel GR agonist-derivatives not affecting osteoblast function.
Taken together, our approach gives new insights into GC action on arthritis and bone that can be translated into new concepts for anti-inflammatory therapies preventing GIO.

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S8.2

Osteoporosis: now and the future

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Osteoporosis is an emerging medical and socioeconomic problem leading to fragility fractures, loss of mobility, chronic pain and cost to the individual and society. Diagnosis is often established only after fracture, although modern techniques exist to make the diagnosis early on where specific therapy is possible. Apart from calcium and vitamin D supplementation and lifestyle counseling, bisphosphonates have become the mainstay of therapy. However, long-term adherence with bisphosphonates is poor and some complications may occur with prolonged use.
The last decade have provided key insights into bone cell biology. Deciphering the regulation of osteoclast differentiation, fusion, activation and resorption led to a comprehensive understanding of osteoclast function and identified potential targets for intervention, including RANK ligand and cathepsin K. Thus, novel molecules such as denosumab, an antibody that inhibits RANK ligand, or odanacatib, a cathepsin K inhibitor have been approved or are in advanced phase 3 studies. Their antiresorptive mode of action differs from that of bisphosphonates and may offer several advantages. With regards to osteoblast biology, characterization of the Wnt signaling pathway provided novel translational prospects to improve local or systemic bone regeneration. More recently, the osteocyte has emerged as a cellular mastermind that senses mechanical strain and guides bone remodeling. The approach to block the endogenous Wnt inhibitor sclerostin with antibodies has emerged as a novel bone-anabolic therapy that is currently evaluated. In conclusion, greater awareness, better prediction models, and novel therapies may change the way of osteoporosis therapy in the future.
Question 1: Which compound is NOT an anti-resorptive osteoporosis drug:
i) Denosumab, an antibody against RANK ligand.
ii) Teriparatide, a PTH analogue.
iii) Zoledronic acid.
iv) Odanacatib, a cathepsin K inhibitor.
v) Raloxifene, a selective estrogen receptor modulator.
Answer 2.

Question 2: Which cells are most common in bone and act as mechanosensors?
i) Osteoblasts.
ii) Osteo-macrophages.
iii) Synovial cells.
iv) Osteoblasts
v) Osteocytes.
Answer 5.

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S8.3

Glucocorticoid-induced osteoporosis

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Osteoporosis is a common complication of oral glucocorticoid therapy and is associated with significant morbidity. Glucocorticoid-induced osteoporosis is characterised by rapid bone loss and increased fracture risk during the first few months of therapy. The increase in fracture risk is dose-related and most prominent in the spine. Although awareness of glucocorticoid-induced osteoporosis has increased in recent years, the condition remains under-diagnosed and under-treated.
Recently there have been advances in fracture risk assessment and treatment in glucocorticoid-induced osteoporosis. The fracture risk algorithm FRAX includes glucocorticoid use as a risk factor but does not take account of the dose, thus underestimating fracture risk in individuals taking higher doses. A modification to FRAX has recently been developed that provides an adjustment for the dose of glucocorticoids.
The rapid increase in fracture risk following initiation of glucocorticoid therapy emphasises the importance of primary prevention of fracture in high-risk individuals. Bisphosphonates are anti-resorptive agents that prevent or reduce glucocorticoid-induced bone loss; vertebral fracture reduction has been demonstrated in secondary analyses. PTH 1–34 peptide (teriparatide) has been shown to increase bone mineral density and reduce vertebral fracture risk in glucocorticoid-treated patients when compared to alendronate treated patients. Bisphosphonates provide the first-line approach in most individuals, with teriparatide as an alternative in those unable to tolerate bisphosphonates. In patients with malabsorption, intravenous zoledronate, 5 mg by i.v. infusion once yearly, is an appropriate option. Calcium and vitamin D supplements should be co-prescribed with these treatments. New recommendations incorporating these advances have recently been issued by the American College of Rheumatology and a joint working group from the International Osteoporosis Foundation and the European Calcified Tissue Society.

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New data treatment of hyperglycemia

S9.1

GLP-1 analogues

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Since 2007 GLP-1 receptor agonists (GLP-1 analogues or incretins) are used for the treatment of type 2 diabetes mellitus. The mechanism of action includes stimulation of insulin secretion from pancreatic β-cells, inhibition of glucagon secretion from α-cells, induction of important β-cell specific genes (e.g. proinsulin, glucokinase and GLUT-2), reduction of postprandial glucose excursions by inhibition of gastric emptying and lowering of overall energy intake via central nervous signaling. In clinical trials they improve glucose control und induce weight-loss in the majority of patients without an increased risk for hypoglycemia. Recently long-acting GLP-1 agonists with a prolonged half-life for once daily or once weekly injection have been developed in order to fully exploit the therapeutic potential of GLP-1 and improve clinical effects. Side effects are similar compared to short-acting GLP-1 receptor agonists, GI-symptoms (nausea and vomiting) seem to occur less frequently. Much interest is focusing on the differentiation of molecules based on the half-life into long-acting versus short-acting GLP-1 receptor agonists, since it is increasingly apparent that exclusively short-acting molecules regulate glucose-metabolism

also by inhibition of gastric emptying while long-acting molecules preferentially act by regulation of islet hormone levels. Thus, the perspective to combine GLP-1 based therapies with basal insulin may be an approach to effectively control both fasting and postprandial glycemia. In long-standing type 2 diabetes basal insulin may be preferentially combined with short-acting GLP-1 receptor agonists, while in early stages of type 2 diabetes long-acting GLP-1 molecules may be used as well. These combination therapies also help to improve individualized diabetes treatment. In order to evaluate the effect of GLP-1 based therapies on clinical endpoints, the results of long-term intervention trials are eagerly awaited.

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S9.2

Abstract for ESE Congress 2013

Early insulin treatment in type 2 diabetes

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The evidence base for starting early insulin treatment in patients with type 2 diabetes is rather scanty. In the UKPDS trial the intensive treatment of hyperglycaemia in newly detected patients with type 2 diabetes based on insulin or sulponylurea (no separation possible) revealed a significant reduction of myocardial infarction risk (15%, $P=0.01$), but only after prolonged post-study follow-up after 10 years[1]. In 2012 the ORIGIN study was published showing no cardiovascular prevention in patients randomized to treatment with an insulin analogue (glargine) as compared to standard therapy[2]. Taken together these two studies do not fully support early insulin treatment in patients with type 2 diabetes for cardiovascular protection. However, other clinical considerations might be of importance, and according to the most recent recommendations from EASD/ADA and their treatment algorithm[3] there may be clinical indications also to support early insulin treatment in these patients not controlled by metformin alone. It should be remembered that some patients with type 2 diabetes in need of very early insulin treatment may instead have late autoimmune diabetes in the adult (LADA) when a diagnosis based on antibodies should be made.

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S9.3

Ultra-long acting insulins

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The limitations of s.c. insulin lead to post prandial hyperglycaemia and a high risk of hypoglycaemia particularly in the post-absorptive state. Nocturnal hypoglycaemia continues to be a common and major problem for patients with insulin treated diabetes and is a major barrier in preventing patients reaching tight glycaemic targets.

The development of basal insulin analogues, insulin glargine and insulin detemir which deliver insulin over a longer period more consistently and with less of a peak effect compared to human insulin are considered a useful advance. Meta-analyses have reported only modest benefit although importantly rates of nocturnal hypoglycaemia are rarely included as an end-point. Nocturnal hypoglycaemia is reduced usefully in many individuals and is a robust indication for the use of these insulins. In some individuals long-acting analogues can be admininstred once daily although many need two injections each day to provide full 24 h coverage.

The development of ultra long acting insulin potentially offers a further advance with universal once a day adminstration and more stable background levels. Although a number of insulins of this type are being developed, only one has been brought to market. Insulin degludec (Tresiba) has been approved for use in Europe and Japan following an extensive clinical development programme. Clinical trials report enouraging and clinically relevant reduced rates of nocturnal hypoglycaemic episodes in patients with either type 1 diabetes or type 2 diabetes. In addition, the precise timing of adminstration is not critical and when given at varying time intervals still leads to stable background concentrations. A single injection of basal insulin in patients with type 1 diabetes brings new challenges in dealing with exercise and other lifestyle issues but ongoing work should provide guidance to clinicians and patients in adjusting insulin and calculating appropriate bolus doses.

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Salt-water balance

S10.1

Abstract unavailable.

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S10.2

Clinical aspects of diabetes insipidus and hyponatremia

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Diabetes insipidus (DI) is a syndrome caused by various defects in the secretion or action of the antidiuretic hormone, arginine vasopressin (AVP). They include impaired AVP production (pituitary DI), increased AVP degradation (gestational DI), suppression of AVP secretion by excessive water intake (primary polydipsia) or decreased antidiuretic effect due to various abnormalities in the kidney (nephrogenic DI). In all four types, the severity of the defect varies between patients. This complicates differential diagnosis by traditional methods based on the urinary response to fluid restriction and injection of AVP or its analogue, desmopressin. An alternative approach is now available. It begins with measurement of basal plasma AVP. If it is normal or elevated (>2 pg/ml), the patient has nephrogenic DI and further testing can focus on the pathogenesis. If basal plasma vasopressin is low (<2 pg/ml), nephrogenic DI is excluded and a brain MRI to determine the absence or presence of the normal posterior pituitary 'bright spot' distinguishes pituitary DI from primary polydipsia. This method is more than 90% accurate as judged by the response to standard therapeutic doses of AVP or desmopressin. Hyponatremia is also due to several different defects in the osmoregulation of antidiuresis. They include impaired suppression of AVP secretion due to non-osmotic stimuli ('effective' hypovolemia, true hypovolemia, nausea or cortisol deficiency) as well as inappropriate secretion of AVP (SIADH) due to ectopic production or primary defects in osmoregulation. The latter take various forms including downward resetting of the osmostat. There is also an AVP-independent type of inappropriate antidiuresis caused by an activating mutation of the V2 receptor or other unidentified abnormalities. Differentiation between the various causes of hyponatremia is a necessary part of the decision whether to treat with hypertonic saline, antiemetic, cortisol or an antagonist of the AVPR2 receptor.

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S10.3

Antidiuresis: insights into the molecular regulation

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The water channel aquaporin-2 (AQP2) of the kidney connecting tubule and collecting ducts, plays an essential role in maintaining body water balance. AQP2 is regulated by the peptide hormone arginine vasopressin (AVP), which exerts parts of its effects through the type 2 vasopressin receptor (AVPR2), expressed throughout the distal nephron. Disrupted function or regulation of AQP2 or the AVPR2 results in nephrogenic diabetes insipidus (NDI). NDI is a common clinical condition of renal origin, which is most often characterized by polydipsia

and polyuria. Major research efforts have advanced our understanding of NDI at the genetic, cellular, molecular, and biological levels. In this talk I provide an overview of the cell biological causes of NDI. In addition, I provide an overview of the new treatment strategies that have been recently proposed for alleviating the symptoms of some forms of NDI, such as those arising from lithium and other drug therapies, acute and chronic renal failure, and disturbed levels of calcium and potassium. I will also discuss cell biological mechanisms for bypassing G protein-coupled receptor signaling.

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New mechanisms in SST analogue response

S11.1

Functional relevance of truncated SST5 receptor variants

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Somatostatin (SST) and its related peptide cortistatin (CORT) exert multiple physiological actions through binding to a family of G-protein coupled receptors (sst1–sst5), commonly bearing seven transmembrane domains (TMDs). However, we have recently discovered that human, pig and rodent sst5 gene also generate, through non-canonical alternative splicing, novel truncated albeit functional sst5 variants that lack one o more TMDs. Our studies indicate that truncated sst5 variants are key elements of the sst family with unique molecular features and functional capacities in mammals, where they can directly interact with long, 7TMD variants (i.e. sst2) and modulate the normal and pathological cellular response to SST/CORT in terms of signaling pathways, hormone secretion and proliferation. Specifically, we have observed that human truncated sst5TMD4 is absent in normal pituitaries and mammary gland tissues but is present in pituitary and breast tumors, in association with long sst5 and/or sst2. In fact, our results indicate that sst5TMD4 might play a relevant patho-physiological role, probably by modulating/impairing the actions of long ssts, which are commonly used as pharmacological targets to treat endocrine/tumoral pathologies (octreotide/lanreotide). Indeed, when coexpressed in the same cell, truncated sst5TMD4 colocalizes and physically interacts with long ssts, providing a molecular basis for the ability of sst5TMD4 to disrupt the normal functioning of sst2/sst5. Moreover, our results indicate that sst5TMD4 is selectively expressed in the most aggressive cases of breast and pituitary tumors, as shown by its correlation with prognostic/proliferative markers. Most importantly, the presence of sst5TMD4 increased malignancy features (i.e. enhanced invasion and/or proliferation abilities) in breast cancer and pituitary tumor cells. Altogether, these results suggest that sst5TMD4 could be involved in the pathophysiology of certain types of tumours, which offer novel avenues to identify and develop original molecular targets for the future diagnosis, prognostic and/or therapeutic treatment of these human pathologies.

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S11.2

Role of filamin A in dopamine and somatostatin receptor targeting in the pituitary

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Molecular mechanisms underlying resistance of pituitary tumors to somatostatin (SS) and dopamine (DA) analogs treatment are not completely understood. Resistance has been associated with defective expression of functional SS (SSTR2–5) and DA (DRD2) receptors. The role of cytoskeleton proteins in receptor trafficking, anchoring to the plasma membrane and signalling has been demonstrated in other tumoral cell systems. In particular, filamin A (FLNA) seems to be a good candidate. In fact, it is implicated in the regulation of DRD2 localization and signalling in different cell types. In particular, we recently demonstrated that both FLNA and DRD2 are strongly reduced or absent in DA-resistant prolactinomas. Moreover, FLNA overexpression in primary cultured cells from resistant prolactinomas restores D2R expression and PRL responsiveness to DA. On the contrary, in cultured cells from DA-sensitive prolactinomas and MMQ cells FLNA silencing resulted in a reduction of D2R expression and abrogation of DA-induced inhibition of PRL release and antiproliferative signals. These data indicate that FLNA is crucial for D2R expression and signaling in lactotrophs, suggesting that the impaired response to DA may be related to the reduction of FLNA expression in DA-resistant prolactinomas.

Since SSTR2 was recently found to associate with FLNA, we investigated a possible role of FLNA in regulating SSTR2 targeting and signaling in GH-secreting adenomas. To this purpose, we performed FLNA silencing in GH-secreting adenoma cultured cells. Preliminary data showed that FLNA silencing did not affect neither SSTR2 localization at the plasma membrane nor its expression stability. On the contrary, the reduction in cyclin D1 levels induced by the selective SSTR2 agonist was abolished in FLNA silenced cells, suggesting that FLNA might be implicated in intracellular signalling of SSTR2 by mediating its antiproliferative effects without affecting receptor expression and localization at the plasma membrane.

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S11.3

Role of ERK in somatostatin receptor signalling

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The Ras/Raf/MEK/ERK is a conserved signalling pathway involved in the control of fundamental cellular processes. Despite extensive research, how this pathway can process a myriad of diverse extracellular inputs into specific biological outcomes is not fully understood. Particularly, the role of this pathway in neuroendocrine tumoral cells remains unclear.

We have previously shown that the ERK1/2 pathway is an integrative point in the control of the pituitary function exerted by various extracellular signals. Moreover we established that the cross talk between the cAMP pathway and the ERK cascade is crucial for the fine regulation of hormonal transcription. In somatotroph tumoral cells, we have shown that the gsp-oncogene (due to an activating mutation of Gsα) induced a sustained activity of ERK1/2 that is involved in the hormonal promoter activation through the GTPases Ras and Rap1. Recently, using the FRET-based biosensors of ERK activity (EKAR), we established that both the EGF receptor and the GPCR coupled to the cAMP-pathway tightly control the spatiotemporal dynamic of activated ERK with different magnitude and duration through the specific recruitment of Ras and Rap1. Moreover, in human pituitary tumors, Dworakowska *et al*. showed that raf/MEK/ERK pathway is up-regulated.

It is well known that octreotide exerts an inhibitory effect on hormonal secretion of pituitary and neuroendocrine cells through the cAMP pathway. A slight but significant inhibitory effect was also observed on tumoral growth and cell proliferation but the signalling mechamisms underlying such an antiproliferative effect remains unclear. Using a somato-lactotroph cell line expressing the sst2 receptor, we identified an opposite effect of ligand-independent and octreotide-dependent sst2 activity on the ERK1/2 activity. The molecular mechamisms involved in SST2-dependent ERK activity is currently under investigation.

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Male reproductive endocrinology

S12.1

Semaphorin 3A: a new gene involved in Kallmann syndrome

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Kallmann syndrome (KS) is a genetic disorder associating pubertal failure with congenital absent or impaired sense of smell. KS is related to defective neuronal development affecting both the migration of olfactory nerve endings and GnRH neurons. The discovery of several genetic mutations responsible for KS led to the identification of signaling pathways involved in these processes, but the mutations so far identified account for only 30–40% of cases of KS. We attempted to identify new KS responsible genes by using a pangenomic approach and we identified SEMA3A. Thus, from an initial cohort of 120 KS patients, we first

studied 48 propositi with no mutations in known KS genes. They were analyzed by CGHarray, using Agilent 105K oligonucleotide chips with a mean resolution of 50 kb. We first found one propositus who had a heterozygous deletion of 213 kb at locus 7q21.11, confirmed by real-time qPCR, deleting 11 of the 17 *SEMA3A* exons. This deletion co-segregated in the propositus' family with the KS phenotype. Later, thanks to a French and European network, additional non synonymous heterozygous mutations in 24 patients, a frameshifting small deletion (D538fsX31) and seven different missense loss of function mutations were reported. *SEMA3A* codes for semaphorin 3A, a protein that interacts with neuropilins. Interestingly, mice lacking semaphorin 3A expression have been showed to have a Kallmann-like phenotype. SEMA3A is therefore a new gene whose loss of function is involved in KS. These findings validate the specific contribution of semaphorin 3A to the development of the olfactory system and in neuronal control of puberty in humans. The mode of inheritance (autosomal dominant or recessive or oligogenic) of this novel genetic form remains to be clearly established in familial and sporadic KS cases. Reproductive phenotypes and gonadotropin axis abnormalities in SEMA3A mutated KS patients and specifically the absence of associated neurological or non neurological clinical disorders will be discussed.

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S12.2

Signal transduction in sperm functions

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Mature spermatozoa acquire progressive motility only after ejaculation. Their journey in the female reproductive tract also includes suppression of progressive motility, reactivation, capacitation, and hyperactivation of motility (whiplash), the mechanisms of which are obscure. MAPKs are key regulatory enzymes in cell signaling, participating in diverse cellular functions such as growth, differentiation, stress, and apoptosis. We have reported that ERK1/2 and p38MAPK are primarily localized to the tail of mature human spermatozoa. Surprisingly, c-Jun N-terminal kinase 1/2 (JNK), which is thought to be ubiquitously expressed, could not be detected in mature human spermatozoa. ERK1/2 stimulation is downstream to protein kinase C (PKC) activation, which is also present in the human sperm tail. ERK1/2 stimulates and p38 inhibits forward and hyperactivated motility, respectively. Hence, a rise in ERK1/2 will induce progressive motility, while a rise in p38 may induce suppression of progressive motility. Concomitant increase in both ERK1/2 and p38 may induce hyperactivation of motility. Both ERK1/2 and p38 MAPK are involved in the acrosome reaction. Using a proteomic approach, we identified ARHGAP6, a RhoGAP, as an ERK substrate in PMA-stimulated human spermatozoa. Inverse correlation was obtained between the relative expression level of ERK1 or the relative activation level of p38 and sperm motility, forward progression motility, sperm morphology, and viability. Therefore, increased expression of ERK1 and activated p38 can predict poor human sperm quality.

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S12.3

Small RNAs in spermatogenesis

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The protection of genetic integrity and establishment and maintenance of correct epigenetic marks are crucial in the germ line to prevent transmission of harmful information to next generations. Male germ cell differentiation is governed by accurate, spatially and temporally controlled gene expression patterns. Male germ cells produce several classes of small non-coding RNAs that are known to regulate gene expression at both post-transcriptional and transcriptional level. These include Dicer-dependent microRNAs (miRNAs) and endogenous small interfering RNAs (endo-siRNAs), and Dicer-independent piwi-interacting RNAs (piRNAs). The functions of these small RNAs in male germ cell-specific gene regulation are still largely unclear. The goal of our research is to clarify the roles of small non-coding RNAs and the mechanisms of post-transcriptional gene control during male germ cell differentiation. We have shown that Dicer is required for spermatogenesis by using a knockout mouse model with Dicer1 deletion specifically in post-natal male germ cells. Our results demonstrate that Dicer and Dicer-dependent small RNAs are crucial for the correct nuclear polarization and chromatin condensation of developing spermatids. piRNAs that are expressed predominantly in the germ line, form a big, complex and functionally diverse group of small RNAs. piRNA expression is known to be greatly induced in late meiotic cells and round spermatids, and interestingly, we have demonstrated that in these cells, piRNAs accumulate in an intriguing cytoplasmic granule, a chromatoid body (CB). We have isolated CBs from mouse testis and characterized its molecular composition to better understand the role of the CB in the post-transcriptional RNA regulation in haploid cells. These pathways appear to be essential for normal spermatogenesis in mouse, thus highlighting their significance in maintaining male fertility.

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Hormonal treatment in transition of patients with rare diseases

S13.1

Hormonal treatment in transition of patients with Prader–Willi syndrome

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Introduction
Prader–Willi syndrome (PWS) is a complex genetic disorder caused by the absence of normal activity in the paternally expressed genes from the chromosome 15q11–q13. PWS is typically characterised by hyperphagia, muscular hypotonia, developmental and cognitive delay, behavioural problems and endocrine abnormalities. Obesity and short stature are common. Controlled studies of hormonal treatment in the transition period are not available.
Endocrine insufficiencies
The combination of the phenotype and reduced GH and IGF1 levels indicates a dysfunction in the GH/IGF1 axis. The degree of GH deficiency varies from mild to severe insufficiency. GH treatment initiated during childhood normalises skeletal growth, improve body composition and optimize mental and motor development. GH treatment with doses normalising IGF1 in adults with PWS improves body composition and to some degree quality of life (QoL) and physical performance. GH treatment is safe but glucose metabolism must be continuously monitored especially in obese patients and in patients with heredity for diabetes, in addition to monitoring serum IGF1 and sleep related breathing disorders.
Incomplete sexual development is frequently seen in PWS. The majority has clinical and laboratory measurements demonstrating hypogonadism, and sex-steroid treatment might be beneficial. Fertility has not been reported in PWS men while five pregnancies have been reported in PWS women.
Central adrenal insufficiency has recently been hypothesized to be responsible of increased risk of sudden death in PWS. Available data indicate that some degree of central adrenal insufficiency may be part of PWS phenotype, although clinically relevant adrenal failure in PWS subjects appears to be rare. Adrenal insufficiency and hydrocortisone treatment should be considered when clinically indicated.
Hypothyroidism is not common in PWS, but TSH and thyroid hormones should be followed regularly.
Conclusion
PWS is associated with documented endocrine insufficiencies which should be monitored and treated.

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S13.2

Transition of females with Turner syndrome

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Treatment with GH during childhood and adolescence allows a considerable gain in adult height. SHOX deficiency explains some of the phenotypic characteristics in TS, principally short stature. Puberty has to be induced in most cases, and female sex hormone replacement therapy should continue during adult years. These issues are normally dealt with by the paediatrician, but once a TS female enters adulthood it is less clear who should be the primary care giver. Morbidity and mortality is increased, especially due to the risk of dissection of the aorta and other cardiovascular diseases, as well as the risk of type 2 diabetes, hypertension, osteoporosis, thyroid disease and other diseases.
The proper dose of hormone replacement therapy (HRT) with female sex steroids

has not been established, and, likewise, benefits and/or drawbacks from HRT have not been thoroughly evaluated. In most countries it seems that the transition period from paediatric to adult care is especially vulnerable and the proper framework for transition has not been established. Likewise, no framework is in place for continuous follow-up during adult years in many countries. Today, most treatment recommendations are based on expert opinion and are unfortunately not evidence based, although more areas, such as GH treatment for increasing height, are well founded.
During the transition period many young females opt out of longitudinal follow-up, probably because they feel well and cannot clearly see the need for continued medical surveillance. However, osteoporosis, diabetes, both type 1 and 2, hypothyroidism, obesity and a host of other endocrinological diseases and conditions are seen more frequently in Turner syndrome in the long term. Prevention, intervention and proper treatment is only just being recognized. Hypertension is frequent and can be a forerunner of cardiovascular disease.
The description of adult life with Turner syndrome has been broadened and medical, social and psychological aspects are being added at a compelling pace. Proper care during adulthood should be studied and a framework for care should be in place, since most morbidity potentially is amenable to intervention.
In summary, Turner syndrome is a condition associated with a number of diseases and conditions which need the attention of a multi-disciplinary team during adulthood.

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S13.3

Hormonal treatment of patients with Klinefelter syndrome during transition

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Hormonal management of androgen, secondary thyroid hormone and vitamin D insufficiency of patients with Klinefelter syndrome (KS) during transition needs some expertise. While vitamin D supplementation should be smoothly applied, thyroxin should be reserved for primary (auto-immune) thyroid disease. Although the androgen deficiency is generally mild and slowly progressive, timely initiation of testosterone replacement therapy (TRT) has been advocated to ensure a normal adult sexual development and to prevent the consequences of long-term androgen deficiency. However, it is unclear if typical complaints and signs of KS, such as decreased facial and body hair, emotional and social developmental delay, eunuchoid body proportions and poor muscle development are fully corrected by TRT. In addition, the efficacy of TRT in the prevention of the metabolic syndrome, varicose veins, leg ulcers, breast cancer and auto-immune diseases is not clearly defined. On the other hand, careful dose adjustment of testosterone is needed to avoid chronic overexposure, given the higher risk of prostate cancer and aggressive behaviour.
Cryopreservation of semen samples or testicular tissue should be considered before initiating TRT. It is unknown whether the suppressive effect of TRT on spermatogenic function is fully reversible and is dose and route of application dependent. On the other hand, testosterone has beneficial effects on semen volume and prostate volume.
Additional therapy with aromatase inhibitors can be considered in patients with gynecomastia, obesity or elevated E2 concentrations or prior to sperm retrieval. Changing dose or route of application of TRT is frequently considered in vain to relieve inherent KS specific physical and psychological problems as chronic fatigue, low vitality, decreased concentration and mood changes. Compliance with daily gel administration is challenging for transition patients. Frequent patient visits may be necessary to maintain and assess compliance, to discuss safety and preventive measures and to avoid discouragement.

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Clinical care of the pheochromocytoma patient

S14.1

Clinical care of the pheochromocytoma patient

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Paragangliomas (PGLs) derive from either sympathetic tissue in adrenal and extra-adrenal locations, or from parasympathetic tissue of the head and neck. Adrenal PGLs are usually referred to as pheochromocytomas. Most patients with adrenal and extra-adrenal abdominal PGLs have increased plasma and urine concentrations of catecholamines (dopamine, norepinephrine and epinephrine). Typical symptoms and signs of catecholamine excess include headache, palpitations, diaphoresis, and hypertension. Although PGL is a rare cause of hypertension, the diagnosis is considered frequently by clinicians in patients with refractory hypertension or symptoms or signs of catecholamine excess.
In about 8–9% of patients with sporadic PGL and 21–31% with hereditary PGL plasma concentrations and urinary outputs of catecholamines are normal. Nevertheless, such patients invariably have elevated plasma concentrations of the metanephrines, normetanephrine and metanephrine. These O-methylated metabolites of norepinephrine and epinephrine are produced continuously within tumor cells and independently of catecholamine release, which can be variable or negligible, even in patients with large tumors. Exceptions where plasma metanephrines can be normal include patients with very small tumors (<1 cm) that do not synthesize and metabolize sufficient amounts of catecholamines to produce positive test results. Other rare exceptions include patients with PGLs that only produce dopamine and which may be detected by increases in plasma methoxytyramine, the O-methylated metabolite of dopamine. In contrast to PGLs derived from sympathetic tissue, the vast majority (95%) of head and neck PGLs do not produce significant amounts of (nor)epinephrine.
At least 30% of the PGLs are caused by germline mutations in ten identified tumor susceptibility genes viz., *RET, NF1, VHL,* succinate dehydrogenase subunits A, B, C, D and assembly factor 2 (*SDHA/B/C/D/AF-2*), *TMEM127 and MAX*. These genotypes correlate with distinct biochemical phenotypes characterized by differences in catecholamine metabolomic and secretory signatures.

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S14.2

Pheochromocytoma imaging

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Pheochromocytomas (PHEO) and paragangliomas (PGL) are neuroendocrine tumors that were born to be imaged using specific radiopharmaceuticals. This is based on the expression of the cell membrane norepinephrine transporter system and somatostatin receptors, as well as GLUT and amino acid transporters found on PHEO/PGL cells.
Anatomical imaging modalities (e.g. CT or MRI) may define the size, shape, structure and enhancement of tumors well, but they lack the ability to explore their functional characteristics. Various functional imaging methods exist, of which positron emission tomography (PET) has become the most popular, in part due to its availability and decreasing cost. PET scanning has the ability to measure tumor characteristics *in vivo,* including such processes as glucose and energy metabolism, DNA and protein synthesis, angiogenesis, apoptosis, hypoxia, and blood flow, among others. Thus, PET in endocrine oncology provides specific tumor detection ('*in vivo*' histology); identifies specific tumor function and targets for therapy; predicts and assesses responses to therapy regardless of tumor size changes ('early' tumor response); may determine the prognosis even before metastatic lesions occur; and eliminates unnecessary treatments, if, for example, a target molecule is not found.
The recent introduction of [^{18}F]fluorodopamine and [^{18}F]fluorodopa as well as [^{68}Ga]DOTATOC and Gluc-Lys[^{18}F]TOCA, together with the previously used [^{18}F]fluorodeoxyglucose, has revolutionized current imaging approaches to PHEO and PGL. The most important results from these studies support the use of [^{18}F]fluorodopamine as the first line of imaging in newly diagnosed PHEO and PGL patients (except in those with head and neck tumors), the use of [^{18}F]fluorodopa as the first line of imaging for SDHB/D- and non-SDHB/D-related head and neck PGLs, and the use of [^{18}F]fluorodeoxyglucose in the assessment of metastatic SDHB-related PHEO or PGL. The newest data also suggests the use of [^{18}F]fluorodeoxyglucose in all metastatic PHEOs and PGLs. [^{123}I]MIBG scintigraphy was found to be suboptimal in PGLs and to perform suboptimally or even poorly, especially in head and neck PGLs. This imaging method performs well in non-metastatic PHEO and is also recommended in those patients in whom [^{131}I]MIBG therapy is planned. Octreoscan has a good value in patients with metastatic PHEO or PGL and in the detection of SDHB-related PHEOs/PGLs in SDHB carriers. PET/CT, rather than PET, has become almost a necessity to perform these studies, and PET/MRI is on the horizon, with some preliminary results already available. The recent use of microCT, MRI, and microPET in an animal model of metastatic PHEO contributed to the introduction of novel therapeutic options awaiting testing in future clinical trials.

Finally, future trends in functional imaging, including its use in pharmacodynamics and molecular imaging, tightly linked to individualized medicine to 'secure' the optimal therapeutic plan, will also be outlined.

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S14.3

Treatment of malignant pheochromocytomas and paragangliomas

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Pheochromocytomas and paragangliomas are rare neuroendocrine chromaffine tumors located in the adrenal or extra-adrenal sites, respectively. Malignant pheochromocytomas or paragangliomas represent 10–20% (MPP) of these patients and are defined by the presence or occurrence of metastasis, in non chromaffin organs. MPP are characterized by their heterogeneous presentation as testified by their variety in primary locations, levels and type of hormone secretions, percentage of genetic disorders and finally frequency of bone metastatic locations. Due to their scarcity, prognostic of MPP have been seldom studied but a hudge heterogeneity in survival has been suggested. SDHB mutation but also the primary location or hormone secretions may constitute critical prognostic parameters. Treatment of MPP has two main goals: the control of hormone-related symptoms and tumor-related burden. In the absence of curative options for advanced MPP patients and randomized trials, the balance between benefits of therapeutic interventions and their safety should be carefully weighed, within skill expert centers and networks, especially in asymptomatic MPP patients with slow rate of progression and low tumor burden. Multiple locoregional options are available to control local progression but also systemic therapies that include meta-iodo-benzyl-guanidine (MIBG) or peptide receptor radionucleide therapy but also, dacarbazine-based-chemotherapy regimen. Recently antiangiogenic therapy has been reported to provide benefit in MPP patients. Protocols are now available for MPP patients, like the first randomized trial in maligant progressive pheochromocytomas or paragangliomas (FIRS-MAPPP) trials in Europe, and can be considered as a first line option in progressive MPP.

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The Frail Male

S15.1

Sarcopenia in men: the endocrine perspective

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The improvement of physical function and mobility in a continuously expanding elderly population emerges as high priority of medicine today. Muscle mass, strength and maximal exercise capacity are major determinant of physical function and all have been shown to decline with aging which may contribute to the increase incidence of frailty and disability observed in elderly men whereas the decline in exercise capacity may facilitate the accumulation of body fat and insulin resistance.

Muscle adaption to exercise seems to be modulated by anabolic circulating endocrine hormones and by autocrine/paracrine local growth factors, which are all load sensitive. GH, IGF1 and T are directly involved in muscle adaptation to exercise as promote muscle protein synthesis whereas T and a locally expressed IGF1 has been reported to activate muscle stem cells called satellite cells. While exercise improves physical function, elderly men fail to sustain an exercise program and a suitable program has yet to be established. GH/IGF1 axis function and T levels decline markedly with aging, which indeed may explain the blunted response of muscle adaptation to exercise observed in elderly men.

Several studies have reported that administration of T improves muscle function in health elderly men. Conversely, GH failed to improve physical function despite the amelioration of detrimental somatic changes of aging. There are evidence of synergistic anabolic action of GH and T and few studies that used this approach have reported greater efficacy. Future studies would need to assess the clinical applicability of these findings as gathering evidence support the indispensable role of endocrine function on physical function integrity.

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S15.2

Male osteoporosis

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Osteoporosis is a silent disorder characterized by reduced bone strength predisposing to increased fracture risk. Although osteoporosis affects women more often than men, ~20% of the 44 million Americans who have osteoporosis or low BMD are men. Between 30 and 40% of fractures due to osteoporosis occur in men; the lifetime risk of fracture for men aged 50 or older is between 13 and 30%. Osteoporosis in men causes significant morbidity and mortality. The major mechanism underlying bone loss with ageing in men is sex hormone deficiency, both testosterone and oestradiol. Men's larger bones are likely a consequence of testosterone's effect on periosteal apposition and this contributes to greater bone strength. There are other reasons why men have fewer fractures than women. Men fall less often than women; higher androgen levels have been associated with reduced fall risk. Finally, men have a shorter life expectancy. The European Society for Endocrinology worked with the Endocrine Society to develop guidelines for male osteoporosis[1]. They recommended testing higher risk men (aged ≥70 and men aged 50–69 who have risk factors (e.g. low body weight, prior fracture as an adult, smoking, etc.)) using central dual-energy X-ray absorptiometry. Laboratory testing should be done to detect contributing causes. Adequate calcium and vitamin D and weight-bearing exercise should be encouraged; smoking and excessive alcohol should be avoided. Pharmacological treatment is recommended for men aged 50 or older who have had spine or hip fractures, those with *T*-scores of −2.5 or below, and men at high risk of fracture based on low bone mineral density and/or clinical risk factors. Treatment should be monitored with serial dual-energy X-ray absorptiometry testing.

Reference

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S15.3

Cognitive decline in male ageing

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Introduction

This is a review of cognitive decline in male ageing.

Methods

Literature review and analysis of cognitive data from the Healthy Old People of Edinburgh (born 1905–1919), Lothian Birth Cohort 1921 and Lothian Birth Cohort 1936 studies.

Results

Cognitive scores in men increased relative to those of women between childhood and late adulthood, but changes in old age were little affected by sex once other factors, such as education, were adjusted for.

Conclusions

Male cognitive ageing differs from women's in early and mid-adulthood, but these differences mostly disappear later in life.

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Oncogenic signals in thyroid cancer - therapeutic prospects

S16.1

$BRAF_{V600E}$ and $PIK3CA_{H1074R}$ cooperate to promote progression of anaplastic thyroid carcinoma in the mouse

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Thyroid malignancies are the most common type of endocrine tumors. Of the various histological sub-types, anaplastic thyroid carcinoma (ATC) represents 2% of all cases but is responsible for most of the thyroid cancer related death. Indeed, ATC is regarded as one of the more aggressive and hard to treat forms of human cancer. Moreover, to date, there is a paucity of relevant models to study how the signature genetic abnormalities detected in ATC contribute to the pathogenesis of this disease.
Mutational activation of the *BRAF* proto-oncogene is detected in ~40% of papillary thyroid cancers (PTC) and also in a significant percentage of ATC. Moreover, *BRAF* mutation is frequently found in combination with gain-of-function mutations in *PIK3CA* (encoding the p110 catalytic subunit of PI3'-kinase-a) or loss-of-function alterations in either *TP53* or *PTEN*. Using mice with conditional, thyrocyte-specific expression of BRAFV600E, we have previously described a model of PTC[1]. However, as in humans, BRAFV600E-induced PTC in mice is indolent and rarely leads to lethal disease.
Using mice carrying a conditional allele of Pik3ca (*lat-1047R*[2]) we demonstrate that expression of mutationally activated PIK3CAH1047R is largely without effect in thyrocytes. However, when BRAFV600E expressed is combined with PIK3CAH1047R in thyrocytes, mice develop ATC that results in rapid lethality. These data indicate that BRAFV600E cooperates with PIK3CAH1074R to promote progression of ATC in the mouse. This genetically relevant mouse model of ATC will be an invaluable platform for testing pathway-targeted therapies for the prevention and treatment of thyroid cancer in the preclinical context.

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S16.2

Abstract unavailable.

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S16.3

Oncogenic activation in thyroid cancer and response to radioiodine

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Metastatic thyroid cancer that are radioactive iodine-refractory (RAIR) have a high mortality, particularly if positive on [(18)F]fluorodeoxyglucose (FDG)-positron emission tomography (PET). We now have a better understanding of the genetic alterations implicated in the disease. Several studies along the last years have shown that constitutive activation of the mitogen-activated protein kinase (MAPK), triggered by rearrangements of RET and NTRK1 and activating mutations of RAS and BRAF, has a causal role in these formation of thyroid tumors. We have recently shown that RAIR, FDG-PET-positive metastases are specially enriched for BRAF mutations. Moreover, when BRAF or RAS are mutated in the primary tumor, it is likely that the metastases will harbor the same alteration. Also, we found that metastatic lesions have alterations in genes of the phosphoinositide 3-kinase (PI3K) pathway such as PIK3CA and AKT1, commonly in concomitance with MAPK activation. In contrast to *BRAF* alterations, *PIK3CA/AKT1* mutations status in different specimens from the same patient are frequently divergent because these mutations arise during progression. These findings are important when considering targeted therapies for metastatic tumors. In fact, the use of the MAPK kinase (MEK) 1 and MEK2 inhibitor selumetinib (AZD6244, ARRY-142886) in patients with metastatic thyroid cancer showed that the drug increases iodine uptake and retention, particularly in *RAS*-mutant patients. Despite the recent advances and increasing knowledge acquired during the last years, a fraction of these aggressive tumors has no identified driver alterations. The use of the most recent sequencing technologies is helping to uncover the additional genetic changes harbored by these patients.

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Medical treatment of endocrine malignancies - an update

S17.1

Pituitary carcinoma

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Pituitary tumours have recently been shown to have a prevalence of around one in a 1000, but the overwhelming majority of these are benign and readily treated. Nevertheless, while the initial therapy of the majority of non-secreting macroadenomas is transsphenoidal surgery, these have a high tendency to recur; recurrence cannot at present be predicted by any histopathological markers. Indeed, some 50% of such adenomas will recur over 10 years, although this recurrence rate is reduced to <5% with standard external beam radiotherapy. Such radiotherapy, while highly effective, does carry the risk of progressive hypopituitarism, but the risk of second tumour development or of visual path abnormalities is very low.
Only around 0.2% of pituitary tumours are carcinomas, as defined by the presence of intra- or extra-cranial metastases, but these offer an exceptional challenge. The majority of these tumours are either prolactin- or ACTH-secreting tumours, and it is interesting that non-functioning and somatotroph adenomas show evidence of 'senescence' markers, which are absent in ACTH- and prolactin-secreting adenomas. Patients with carcinomas may occasionally respond to dopamine- or somatostatin-receptor agonists, but in general require repeated transsphenoidal or even transcranial surgery. Conventional chemotherapy is generally ineffective, but recent reports with the alkylating agent temozolomide have shown cases of impressive tumour control, at least in the short term. The enzyme MGMT reverses the effect of temozolomide by removing the methyl adduct from DNA, and reports suggest that tumours lacking MGMT are especially sensitive to temozolomide. However, an extensive study has shown that only some 15% of pituitary adenomas lack MGMT, and it is probably reasonable to consider temozolomide therapy for 3–6 months regardless of MGMT status, which is in any case difficult to quantify. In theory, tumours which remain progressive following temozolomide may respond to everolimus, an mTOR inhibitor, but data on this are scanty.

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S17.2

Medical treatment of adrenocortical cancer

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Adrenocortical cancer (ACC) is a rare tumor with an overall poor prognosis. However outcome is heterogeneous and the indication and type of medical therapy should be personalized on the basis of tumor stage and prognostication. The initial diagnostic and extension work-up of an ACC is an important step. Most patients with ENSAT stage 1–3 ACC and some stage 4 ACC will be operated and pathological analysis is important for management. The goals of medical treatment are to control steroid excess and tumor development. Symptomatic treatment and steroidogenesis inhibitors can be used to control the consequences of secreting ACC. Mitotane is usually the drug of choice for both its anticortisolic and adrenolytic effects. However, due to its long delay of action it can be associated in severe cortisol secreting ACC with other drugs like metyrapone and/or ketoconazole, which have a faster effect. Mitotane can be also discussed as adjuvant therapy after complete removal of a tumor for its adrenolytic effect to prevent tumor recurrence. Retrospectives studies have shown a longer disease free survival with adjuvant mitotane, but this has not be a constant finding. One should keep in mind that the probability of recurrence varies among stage 1 and 2 ACC and that development of pathological or molecular

markers for risk assessment will help. In case of tumor recurrence or in metastasized ACC, medical therapy with mitotane is used and adapted to its plasma levels. Cytotoxic chemotherapy will be discussed depending on the rate of tumor progression and the response to mitotane. The FIRM-ACT trial has shown, in patients treated with mitotane, a better response rate and progression free survival using etoposide-doxorubicine-cisplatine than streptozotocine. Others cytotoxic chemotherapies and targeted therapies have been used in progressive ACC with lower response rate and will be summarized. Since most ACC overexpress IGF2 the IGFs inhibitors have been developed. International efforts in prospective therapeutic trials are developed in this field. For this goal patient management in coordination with expert centers and networks of clinical research (in Europe www.ensat.org) are important.

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S17.3

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PCOS

S18.1

Environmental impact on the PCOS phenotype

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The action of insulin acting as a co-gonadotrophin in women with PCOS has long been a focus of research. In western populations, a key environmental effect on PCOS is through the obesity epidemic. The resulting insulin resistance has direct effects on the clinical manifestation of PCOS with serum insulin showing positive associations with BMI, serum testosterone, AMH and variably with ovarian volume. AMH is a particularly interesting marker of PCOS with respect to insulin as diverse results have been found with BMI where associations have been found to be either negative or non-existent.
The characteristics of South Asian women with PCOS has been a point of interest over recent years because of the propensity to insulin resistance and type 2 diabetes in this population. While the prevalence of metabolic syndrome has been well documented, relatively little is known about the ovarian response to insulin status in women with PCOS in this region. South Asian women with PCOS have relatively mean low BMI and it is possible to explore the effects of insulin resistance at a lower end of the spectrum than is possible in many western cohorts. The nature of development in much of India provides an interesting model to explore the effects of environment on PCOS. In rural areas women take a simple diet of millet and undertake physical labour in agriculture. There is then a spectrum of diet and activity through to an urbanised group who have a considerable intake of refined carbohydrate and 'junk food' and who take little exercise. Preliminary data from India shows a major impact from life style on several ovarian markers in PCOS including serum AMH concentrations.

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S18.2

PCOS in adolescence: towards a therapy targeting adipose tissue

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PCOS is a common endocrinopathy in women that has traditionally been viewed as an ovarian disorder. Accordingly, the classic therapeutic approach, even in adolescents without pregnancy risk, is to silence the ovaries with an oral contraceptive (OC). Recent evidence indicates that PCOS is primarily a disorder of adipose-tissue hyperexpansion that may originate in early life, develop across childhood and puberty, and reach a full-blown stage in adolescence, manifesting with ovulatory dysfunction and symptoms of androgen excess. This novel concept implies that intervention should rather aim at reducing adipose-tissue hyperexpansion, and thus at correcting the associated insulin resistance, visceral adiposity and low-grade inflammation.
In non-obese PCOS adolescents without pregnancy risk, a low-dose combination of flutamide (Flu, an androgen-receptor blocker) and metformin (Met, an insulin-sensitizer) proved superior to a drospirenone-OC in correcting the endocrine-metabolic and body-composition anomalies. In young PCOS women receiving OCs, the addition of low-dose pioglitazone (Pio), to the FluMet combination further increased lean body mass and HMW-adiponectin, and further reduced carotid intima-media thickness (cIMT).
Recently, we compared the effects of low-dose PioFluMet to those of an OC containing ethinylestradiol-cyproteroneacetate (EE-CA). Both treatments reduced androgen excess similarly, but had divergent effects on glucose-induced insulinemia, visceral adiposity, low-grade inflammation, cIMT, menstrual regularity, and on the expression in subcutaneous fat of genes related to macrophage activation, fat accretion, inflammation, and lipoprotein metabolism. These divergences were to the advantage of PioFluMet, and were still evident 6 mo post-treatment.
In conclusion, the intervention for reducing androgen excess in adolescence influences the post-treatment phenotype, as judged by markers of insulin sensitivity, visceral adiposity, arterial health, low-grade inflammation and menstrual regularity. A novel low-dose combination of insulin sensitizers plus an anti-androgen holds potential as a pathophysiology-based treatment of PCOS in adolescence and prevents part of the androgen-excess phenotype in adulthood, including adiposity and subfertility.

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S18.3

The diagnosis of PCOS in adults: new biomarkers

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The National Institute of Health (US) recently organized an Evidence-based Methodology Workshop on the polycystic ovary syndrome (PCOS), focused on definition of the syndrome. Among others, the conclusions included the following: i) the need to assign a name that reflects the complex interactions that characterize the syndrome; ii) it is advisable to maintain a broad, inclusionary diagnostic criteria of Rotterdam, while specifically identifying the most common phenotype, characterized by androgen excess and ovulatory dysfunction; iii) the need to improve the methods and criteria used to assess androgen excess and develop an accurate assay for androgen levels; iv) it is necessary to improve the methods and criteria used to assess ovulatory dysfunction; v) it is necessary to investigate whether specific phenotypes of PCOS are associated with increased CV and diabetic complications. These recommendations intriguingly imply the need for further biomarkers. Potential biomarkers of ovarian dysfunction might be the measurement of blood levels of anti-Mullerian hormone and insulin like factor 3. The use of LM-MS/MS provides an important improvement in the assay of androgens. The combination of an extremely useful clinical parameter, acanthosis nigricans, combined with adiponectin and insulin blood levels may help in defining the presence of insulin resistance. Low sex hormone-binding globulin may be a marker of insulin and androgen excess and might predict the susceptibility to develop type 2 diabetes. Finally, consistent with the available literature on the role of low-grade inflammation in the pathophysiology of androgen excess and metabolic disturbances, measuring specific markers such as TNFα may help in characterizing specific phenotypes.

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Recent advances in the molecular study of endocrine tumours: microRNAs and more

S19.1

TGF-β signaling and the microRNA machinery

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The activin/transforming growth factor-β (TGF-β) is a family of evolutionary conserved polypeptides. Their role has been implicated in the regulation of embryonic development, reproduction and tumor formation.
Related to tumorigenesis TGF-β signaling inhibits cell proliferation at multiple levels by i) inducing the expression of tumor suppressors $p15^{Ink4b}$ and $p21^{Waf1}$, ii) repressing oncogenic factors such as c-MYC and Id proteins, iii) by activation apoptosis and iv) inhibiting tumor growth by repressing hepatocyte growth factor (HGF), macrophage-stimulating protein (MSP) and TGF-α. Loss of members of

the TGF-β signaling by somatic mutations or epigenetic events, such as DNA methylation or regulation by microRNA (miRNA) have been demonstrated to affect the signaling process. MmiRNAs are 19–25 nucleotides long, non-coding RNA molecules that posttranscriptionally regulate gene expression via RNA interference. At least 30–50% of all protein-coding genes are under regulation of miRNAs. Their expression is highly tissue-specific, and one miRNA affects the expression of several proteins and vice versa one protein is influenced by several miRNAs. Their role has been revealed in several physiological and pathological cellular processes including development, cell proliferation, differentiation, apoptosis and tumorigenesis. Therefore, this complex bidirectional link makes the mRNA-miRNA couples as an ideal targets for various therapuetical approaches. In addition, the biogenesis of miRNAs is also regulated by TGF-β, by regulating the maturation process of miRNAs. Members of TGF-β signaling are also targets for miRNAs. Hence auto-regulatory feedback loops between TGF-β and miRNAs influence the fate of tumor cells. Our aim is to review the crosstalk between TGF-β signaling and the miRNA machinery in order to discover bidirectional feed-back loops which contribute to the tumorigenesis process of endocrine glands, and to identify potential novel therapeutic targets.

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S19.2

MicroRNAs in pituitary tumours

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MicroRNA (miRNAs) are small non-coding RNA molecules that significantly impacted the understanding of cell biology in the last decade. They regulate the expression of 10–30% of all protein-coding gene, thus playing a crucial role in a wide range of biological and pathological processes. Consistently, aberrant miRNA expression has been implicated in numerous human diseases, including pituitary tumours. Different studies analysed the entire miRNA transcriptoma, by microarray and RT-PCR, in normal and neoplastic pituitary tissues, and several aberrant expression patterns of miRNAs in pituitary tumours as compared to normal pituitary have been identified to date. A different miRNA signature has revealed miRNAs to be useful for discrimination of the different pituitary adenoma sub-types, for distinguishing pharmacological treated pituitary adenomas, as well as for differentiation of microadenomas from macroadenomas and carcinomas from adenomas. This is particularly important in the still unsolved issue of distinguishing benign from malignant pituitary tumours prior to metastasis. Based on their differential expression, it is possible to infer the likely role of certain miRNAs in tumor development. In this way a number of studies identified miRNAs controlling pituitary cell proliferation, apoptosis and invasion. Also by identifying the putative or confirmed target of the aberrantly expressed miRNAs has been possible to get significant insight into the role of miRNAs in pituitary tumorigenesis. This is the case of a group of miRNAs, all downregulated in a wide range of pituitary adenomas compared to normal pituitary tissue, which are able to inhibit pituitary cell growth by targeting HMGA proteins in pituitary cells. Indeed, overexpression of HMGA genes is a common feature of human pituitary tumours and it causes pituitary cell cycle dysregulation and development of pituitary adenomas in transgenic mice, thus suggesting that HMGA genes might be considered as specific oncogenes for pituitary cell transformation. Therefore, the restoration of miRNAs targeting HMGA genes or other pituitary oncogenes may represent a new promising therapeutic approach for pituitary adenomas.

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S19.3

MicroRNAs and gene expression patterns in adrenal tumours

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MicroRNA and mRNA expression patterns have been investigated in several studies on adrenocortical tumours and in some reports on phaeochromocytomas. These studies are relevant from pathogenic, diagnostic and therapeutic aspects. Under- and overexpressed microRNAs representing tumour suppressor and oncogenic microRNAs have been reported that can be helpful in the diagnosis for differentiating benign and malignant adrenocortical tumours (e.g. miR-503-miR-511, miR-195, miR-483-5p), recurrence-prone and non-recurring phaeochromocytomas (miR-1225-3p) and even benign and metastasing phaeochromocytoma (e.g. miR-15a, miR-16, miR-101, miR-183). Differentially expressed mRNAs and microRNAs have been identified that confer prognostic information. Based on mRNA expression patterns, adrenocortical cancer can be subdivided in two groups with different prognosis. Analysis of pathways affected by mRNAs and microRNAs has revealed several potentially druggable pathomechanisms e.g. mTor in adrenocortical tumours, Notch-signaling in phaeochromocytomas. Since the drug repertoire for treating adrenocortical cancer and malignant phaeochromocytoma is rather limited, these novel bioinformatics approaches might be of great relevance for deciphering novel pathways. By network analysis, we have found the underexpression of c-MYC in adrenocortical cancer that might be a central pathogenic event. Meta-analysis of adrenocortical tumour genomics data revealed three major pathomechanisms including: i) damage of cell cycle, ii) retinoic acid signalling via retinoid X receptor, iii) immune alterations. Based on these observations, our *in vitro* studies on 9-*cis*-retinoic acid in the adrenocortical cancer cell line NCI-H295R have shown the inhibition of growth and hormone secretion, and robust changes in gene expression patterns. 9-*cis*-retinoic acid also inhibited tumour growth *in vivo* in a xenograft model that might raise its potential applicability in the treatment of adrenocortical cancer. These findings may pave the way for novel diagnostic and even individualized treatment protocols.

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New mechanisms of energy balance

S20.1

Abstract unavailable.

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S20.2

Programming of host metabolism by the gut microbiota

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The gut microbiota has co-evolved with its mammalian host and is an important factor that contributes to host metabolism. Metagenomic analyses of stool samples from patients have revealed that the gut microbiota is altered in these metabolic diseases and germ-free mice are protected against disease development. Thus, the gut microbiota has been implicated as a causative, or contributing, factor to obesity, diabetes as well as cardiovascular disease. By using germ-free and conventionally raised mice we have demonstrated that the gut microbiota signals through FXR, a nuclear receptor, by removing a natural occurring FXR antagonist. FXR is an important regulator of host metabolism and microbial activation of FXR may provide a mechanism by which the gut microbiota modulates obesity and diabetes. Furthermore the gut microbiota is in close interactions with enteroendocrine cells in the gut and may affect their function through signaling by G-coupled receptors. Effects on gut microbiota on selected gut derived hormones will be discussed.

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S20.3

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Multi-centre pituitary studies

S21.1

New classification of pituitary tumours based on the hypopronos database

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Pituitary endocrine tumours are currently classified by histological, immunocytochemical and numerous ultrastructural characteristics but without prognostic clinical correlations. We investigated the prognostic value of a new clinicopathological classification with grades based on invasion and proliferation, the components of tumour behaviour.
This retrospective multicentric case–control study comprised 410 patients who had surgery for a pituitary tumour with long-term follow-up. Using pituitary magnetic resonance imaging for diagnosis of cavernous or sphenoid sinus invasion, immunocytochemistry, markers of the cell cycle (Ki-67, mitosis) and p53, the tumours were classified according to size (micro, macro and giant), type (PRL, GH, FSH/LH, ACTH and TSH) and grade (grade1a: non-invasive; 1b: non-invasive and proliferative; 2a: invasive; 2b: invasive and proliferative; and 3: metastatic). The association between patient status at 8 years follow-up and age, sex, and classification was evaluated by two multivariate analyses assessing disease-free or recurrence/progression-free status.
At 8 years after surgery, 195 patients were disease-free (controls) and 215 patients were not (cases). In 125 of the cases the tumours had recurred or progressed. Analyses of disease-free and recurrence/progression status revealed the significant prognostic value ($P<0.001$; $P<0.05$) of age, tumour type, and grade across all tumour types and for each tumour type. Invasive and proliferative tumours (grade 2b) had a poor prognosis; with an increased probability of tumour persistence or progression of 25- or 12-fold respectively compared to non-invasive tumours (grade 1a).
This new, easy to use clinicopathological classification of pituitary endocrine tumours has demonstrated its prognostic worth by strongly predicting the probability of post-operative complete remission or tumour progression and so could help clinicians choose the best post-operative therapy.

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S21.2

Lessons from the Liège Acromegaly Survey (LAS)

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The Liège Acromegaly Survey is a cross-sectional study on acromegalic patients, developed as an in-house tool in Liège, then extended to other Europeans participating centers. The database differentiates itself from national and multinational registries by adopting a goal oriented approach and it was conceived following a list of open questions on acromegaly. After an initial test run in Liège, the database installation started on mid-March 2010. Thirteen other european centers joined the project. At this time, 3050 patients from fourteen centers were included.
Sex ratio (M/F) was 1397/1653 ($=0.85$) with strong differences between centers (Porto: 0.43, Valencia: 1.4). Median age at diagnosis was 45.1 and the male population younger than the female (medians: M$=$43.4, F$=$46.2, $P<0.001$). When looking at patients' age and the year of diagnosis, a trend toward an aging of the population was observed with more older patients getting diagnosed with time.
Median duration of disease before diagnosis was 5 years whenever estimation was possible. Acromegaly was suspected in 43% of cases by endocrinologists, in 16% of cases by an internist from different specialities and in 17% of cases by family doctor. Dysmorphia appears as the main sign leading to diagnosis.
At diagnosis, glucose levels in non-diabetic patients, hemoglobin concentrations and red blood cell count correlated with IGF1 levels but not GH, suggesting that IGF1 values are a better representation of the activity of acromegaly than GH.
In summary, the LAS was conceived as a tool to answer a number of questions on the subject of acromegaly. By concentrating on these specific points and recording data from a more than 3000 patients, this database seems to be able to highlight a number of aspects not addressed before.

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S21.3

Lessons from the European Cushing's registry (ERCUSYN)

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ERCUSYN owned by ESE, was set up in 2007 with European funding for 3 years. Sustainability has been achieved by agreements with pharma, and generous support from funding partners and Lohmann and Birkner GmBH, who established and maintained the on-line registry and web (www.ercusyn.eu).
On-line brochures for patients and physicians are available in different languages, as well as details on >50 participating centres in 28 European countries. By 2012 over 800 patients are included. Data definitions in detailed guidelines are available on-line. Ethical and legal considerations respecting individual requirements in each centre or country have been introduced.
ERCUSYN has evidenced a heterogeneous clinical presentation of CS, depending on gender and aetiology, and confirms long delays between onset of symptoms and diagnosis, with many specialists consulted who often missed the correct diagnosis. Morbidity at diagnosis is high, with low bone mass, especially in men, and impaired quality of life. Less than half the cohort was actively working, despite a mean age of 44 years. So there is great potential for improvements in the delay to diagnosis, with obvious beneficial consequences both for patients and for the health care systems, due to long-term consequences of delayed diagnosis and increased morbidity.
ERCUSYN represents the largest prospective collaboration of CS in Europe, with potential for improving the care of patients, and may be used as a rare disease registry for new orphan drugs to be evaluated. It can be liaised to the European Medicinal Agency-regulated, industry-required post-marketing surveillance studies, to follow safety and efficacy in the long-term outcomes in clinical practice conditions. Through the ESE this network may be used to disseminate information and encourage further interaction between endocrinologists across Europe.
Reference
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Improving diagnosis of primary aldosteronism

S22.1

Genetics of primary aldosteronism

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Excessive autonomous aldosterone secretion by the adrenal gland, so called primary aldosteronism (PA), causes drug-resistant and often life threatening arterial hypertension accompanied by severe hypokalemia. Long-term consequences include increased risk for stroke, myocardial infarction and atrial fibrillation. PA is present in up to $\sim$10% in referred patients and 7% in primary care, but as high as 20% in patients with resistant hypertension. The early detection of PA has clear impact on clinical outcome and survival, given the major cardiovascular adverse effects of aldosterone excess, which are independent of blood pressure, and predict outcome following surgical.
With an increasing pace genetic mechanisms have been identified that contribute to autonomous aldosterone secretion. These include both familial forms as well as acquired somatic cases which partly overlap. Familial hyperaldosteronism type I (FH-1), also called glucocorticoid suppressible aldosteronism, is characterized by early and severe hypertension with biochemical abnormalities of PA and production of hybrid steroids. Fusion of the promoter region of CYP11B1 to the coding region of CYP11B2 produces a chimeric gene, with activity of aldosterone synthase, but regulatory specificity of that of 11β hydroxylase, which results in the synthesis of aldosterone under control of ACTH. FH-2 has an autosomal dominant mode of inheritance with a variable phenotype including APA and BAH even within the same family. Clinical and biological features of FH-2 are indistinguishable from that of sporadic PA. While in one large family a locus associated with the disease has been mapped to chromosome 7p22 no underlying genetic mutation has been resolved. Recently, exome sequencing in sporadic APAs has revealed somatic mutations in the KCNJ5 gene which is present in up to

30% of cases. Furthermore, some rare familial cases with germ-line mutations of KCNJ5 have been described and attributed to FH-3. This condition is responsible for increased calcium influx into the glomerulosa cell leading to constitutive secretion of aldosterone and possibly cell proliferation.
Recently, a number of approaches including genome wide association studies, exome sequencing and mutagenesis screens have been applied to patient cohorts and *in vivo* models of PA. Thereby, it is likely that in the near future further pathophysiological mechanisms that result in autonomous aldosterone secretion will be unraveled. Combination of genetic marker with clinical annotations from prospective studies will be required to allow for disease stratification and individualized treatment decisions for affected patients.

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S22.2

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S22.3

Adrenal vein sampling

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Primary aldosteronism is the most common form of secondary hypertension. The detection of primary aldosteronism is of particular importance, because it provides an opportunity for a targeted treatment (surgical for unilateral forms and medical with mineralocorticoid receptor antagonists for bilateral forms). PA diagnosis comprises screening, confirmation testing and subtype diagnosis.
Guidelines recommend that all patients for whom the surgical treatment is practicable and desired should undergo adrenal vein sampling (AVS) as the gold standard to differentiate unilateral from bilateral adrenal disease. AVS is technically difficult, especially in terms of successful cannulation of the right adrenal vein and the success rate increases with experience and dedication of the radiologist. The introduction of a method for real-time rapid cortisol assay during AVS, which provides the radiologist of information on the success of the cannulation, offers a new tool to improve the performance of AVS. We recently assessed the impact of differing criteria for successful cannulation and lateralization on reproducibility of subtype diagnosis and also the effect of ACTH stimulation. The use of permissive cannulation success criteria can lead to significant numbers of patients being given a definitive subtype diagnosis when stricter criteria would have led to the study results being rejected or interpreted with great caution; unfortunately, permissive criteria are associated with a low reproducibility of the diagnosis. Furthermore, we showed that ACTH infusion may be of help for those centers with a low rate of cannulation and perform at least as well as the unstimulated protocol for final diagnosis of PA subtypes. The possibility of performing adrenalectomy in some selected PA patients without AVS and the necessity of having contralateral suppression of the aldosterone secretion from the adrenals, is still a matter of discussion.

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Endocrine disruptors

S23.1

Abstract unavailable.

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S23.2

Male reproductive health and endocrine disrupters

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There are robust data from all parts of the World that the incidence of testicular germ cell cancer, which is often associated with dysgenesis and poor spermatogenesis in adjacent 'normal' testicular tissue, has been increasing during the past 50 years. There is also evidence of widespread poor semen quality among young men in European countries, where there also have been reports on declining semen quality. Although the latter findings have been subject to controversy, recent large studies of young men from Denmark, Germany, Spain, France, Finland and Japan have shown that 10–15% of the men had sperm concentrations at levels indicating a high risk of need of assisted reproduction in case they wanted to father a child, and an even larger group of young men in these countries seem to be at risk of a prolonged waiting time to pregnancy. In addition, cryptorchidism and hypospadias remain among the most common congenital abnormalities among boys. These trends taken together with recent reports, showing that 8% of birth cohorts are now born after assisted reproduction, indicate that male infertility may significantly contribute to the decline in fertility rates seen in Europe and many Asian countries. A hypothesis will be presented that modern lifestyle with its wide exposure to endocrine disrupters may play a role together with adverse effects of smoking and obesity.

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S23.3

Are structural analogs to bisphenol A a safe alternative?

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Bispenol A (BPA) is a chemical often integrated in impact-resistant plastics and surface coatings in canned foods as well as screw-on caps and cashier receipts. BPA is either intentionally added or appear as a consequence of recycling in materials with food contact, which leaves consumers at risk of exposure. BPA has known endocrine disrupting effects and is suspected to be a contributing factor in disorders such as overweight, diabetes, cardiovascular diseases, and behavioral changes in children. Therefore, developing alternatives to BPA is important.
The aim of this study was to characterize the toxicological profile of BPA and five analogs, BPB, BPE, BPF, BPS, and 4-cumyl phenol with focus on general toxicity and endocrine disrupting potential. The investigation was conducted by performing a comprehensive quantitative structure activity relationships (QSAR) modeling with respect to a series of adverse human effects, including acute oral toxicity, local irritation, cardiotoxicity, endocrine disruption, reproductive toxicity, genotoxicity, and cancer. Their metabolism via three key cytochrome P450 enzymes and the pregnane X receptor was also evaluated.
The toxicological profile of the six bisphenols were supplemented with and compared to data obtained from experimental cell-based assays covering interferences with the androgen, estrogen, PPARγ, and aryl hydrocarbon receptors and interferences with steroidal sex hormone synthesis.
Overall, the qualitative profile was in general similar. However, important quantitative differences in potency and efficacy appeared. Work is in progress to reach the overall conclusions.

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Redefining our understanding of the causes of obesity

S24.1

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S24.2

Social stress, obesity and type 2 diabetes

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Chronic stress, low socioeconomic status and ingestion of hypercaloric food are all recognized risk factors for obesity, metabolic syndrome (MetS) and type 2 diabetes (T2D). Given the complexity of these metabolic processes and the unavailability of animal models, there is poor understanding of their underlying mechanisms. We established a model of chronic psychosocial stress (CPS) in which animals of low social rank (subordinates, SUB) are vulnerable to weight gain while animals of high social rank (dominants, DOM) are resilient. Recent data will be discussed demonstrating that social status and genetic predisposing factors interact with the nutritional environment to establish individual vulnerability to stress-induced metabolic disorders. Firstly we demonstrated that animals exhibiting high rank (DOM) that are hyperactive and show sympathetic hyperactivity, are fully protected from the development of metabolic disorders when fed both a standard and a high fat diet, despite being hyperphagic. Secondly, we demonstrated that SUB mice fed a standard diet exhibited features of MetS and downregulation of the insulin pathway downstream of IRS and PPARs in liver and skeletal muscle as well as complex changes in lipid metabolism facilitating fat deposition and in WAT. Furthermore, exposing wt mice to hypercaloric diet induced the development of glucose intolerance and insulin resistance. Similarly subordination stress aggravated glucose intolerance in the diabetic db/db strain of mice. Overall, we demonstrated a robust stress- and social status-dependent effect on the development of MetS and T2D and provided insights on the underling molecular mechanisms. Our results are reminiscent of, and provide a model for, the effect of the individual socioeconomic status on human health.

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S24.3

Ambient temperature and other environmental factors in obesity

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Discussion of environmental factors in the development and maintenance of obesity has overwhelmingly focused on the effects of an energy-dense and plentiful food supply and an environment conducive to physical inactivity. In recent years, however, an increased understanding of epigenetics has led to an explosion of interest in the mechanisms through which environmental exposures can exert an influence on energy intake and energy expenditure. Upward trends in domestic winter indoor temperatures is one such influence. Plausible causal mechanisms have been suggested for an effect of reduced exposure to mild cold on population increases in obesity. Experimental studies confirm that human energy expenditure is associated with temperature exposures within the range relevant to indoor heating trends, and the existence of variable amounts of cold-activated brown adipose tissue in adult humans has raised the possibility that thermogenic capacity might be adversely affected by reduced seasonal cold exposure. While these pathways are plausible their validity has not yet been demonstrated in free-living humans. As with other putative environmental factors, translating laboratory research to a real world setting presents a substantial challenge and there are many outstanding questions around the triggering of biological and behavioural compensation mechanisms. Nonetheless the study of the role of environmental factors in body weight raises exciting possibiliies for novel public health strategies to address obesity.

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Rare metabolic bone disease

S25.1

Paget's disease of bone: how to treat and monitor patients

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Paget's disease of bone (PDB) is the second commonest bone disease. The original disease description in 1876 by Sir James Paget remains an incisive, accurate report of the pathophysiology of PDB, which is characterised by focal regions of increased bone remodelling with initial excessive bone resorption and osteolytic lesions followed by long term increased bone formation and sclerosis. The majority of patients are elderly, many are asymptomatic. Those that are referred to hospital are usually symptomatic with bone pain, skeletal deformity, pathological fractures, neurological symptoms and deafness[1].

There is marked geographical variation in PDB with the highest prevalence/incidence in the UK, particular North West England. Recent studies in the UK and New Zealand have suggested that there is a decline in the prevalence and severity of PDB and a gradual decrease in concentration of total ALP at presentation[1–3].

Measurement of new biochemical markers of bone metabolism have failed to establish an obvious successor to total ALP in terms of the cost-benefit for the information gained either for diagnosis or follow-up. The modest benefit of bone specific ALP is seen in patients with monostotic (single bone involvement) PDB.

The bisphosphonate drugs are the treatment of choice for PDB. IV zoledronate has been assessed compared to oral risedronate in 176 patients with PDB. A single 5 mg infusion of zoledronate produced a more rapid, more complete, and more sustained response in PDB than 2 months treatment with 30 mg risedronate daily[4,5].

However questions have been raised regarding the optimal way to treat and assess patients with PDB. A prospective randomised trial of intensive vs symptomatic management (PRISM) of PDB has been performed to investigate how patients should be treated and subsequently extended to include treatment with zoledronate (PRISM-EZ). 1324 entered PRISM with one half receiving increasing analgesia or mild bisphosphonate treatment (symptomatic) and the other group receiving potent bisphosphonates to maintain their total ALP within the reference range (intensive). The primary endpoint was fracture, with secondary endpoints of progression of deafness, requirement for orthopaedic surgery and quality of life[6]. No significant difference was observed between the treatments after an average of 32 months treatment.

There is some suggestion from these recent trials that the previous apparent close association between total ALP and disease activity/symptomatology, especially following treatment, no longer holds true and patients should always be assessed clinically including radiological assessment rather than basing treatment purely on biochemical measurements[6].

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S25.2

Osteogenesis imperfecta

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Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder with a prevalence of 1/15 000 to 1/20 000 individuals. In more than 90% of patients with type I–IV OI, the disorder is due to a dominant mutation in one of the two genes that encode the α chains of collagen type I, Col1A1 and Col1A2. Collagen type I is present in many tissues, and in addition to multiple fractures patients with OI can have dentinogenesis imperfecta, blue sclera, hyper mobile ligaments and skin, hearing loss and increased risk of bruising. Classically, OI is divided into four subtypes, Sillence class I–IV, with the addition of three more rare forms. Type I is the most common and is generally mild and non-deforming. Type II is the most severe and is lethal already in the perinatal period, usually due to respiratory insufficiency from multiple thoracic fractures. Type III is severely

deforming and patients often have fractures at birth. Type IV has a clinical range between type I and III and thus is moderately deforming. Recently described types V–VII are rare and lead to a moderate to severe phenotype. The majority of patients with OI have a low BMD.
To date there is no cure for OI. Many patients are treated with bisphosphonates, which there is support for in clinical trials, most of these are in children with OI. It is not known if treatment with other osteoporosis drugs would be a better alternative or a perhaps a complement to bisphosponates in the patient with OI. A therapeutic vision is to use either stem cell therrapy or silencing of the mutated genes through RNA interference (RNAi).
In this presentation the litterature concerning effects of antiosteoporotic drugs in adult patients with OI will be revived.

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S25.3

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Novel technologies and inspiring ideas: From basic endocrine research to clinical practice (European Young Endocrine Scientists (EYES) Symposium)

S26.1

Making a functional thyroid in a dish

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During the last decade induced overexpression of defined transcription factors has been shown to have a driving effect on the differentiation of embryonic stem cells (ESCs) into many specific cell types. Nevertheless, the generation of protocols promoting a coordinate self-assembly of differentiated cells into distinct morphological units with also functional properties reminiscent of organs and tissues *in vivo* are still very sparse. Recently, we have reported the generation of functional thyroid follicles generated from pluripotent stem cells. We show that a transient overexpression of the transcription factors NKX2.1 and PAX8, notably know to play a pivotal role during thyroid organogenesis, in addition to a subsequent treatment with TSH is sufficient to direct murine ESC differentiation into thyroid follicular cells (TFC) and promotes *in vitro* self-assembly of TFC into three-dimensional follicular structures. In addition to morphological evidences, cells differentiated by this protocol showed significant iodide organification activity, a second hallmark of thyroid tissue function. Importantly, athyroid mice grafted with mESC-derived thyroid follicles show normalization of plasma T_4 levels with concomitant decrease of plasma TSH. Moreover, normalization of body temperature had been observed upon transplantation of thyroid follicles into athyroid mice. Our findings have demonstrated that mESCs can efficiently be differentiated into TFCs that can self-assembly into 3D functional units and finally transplanted in athyroid mice rescuing the hypothyroid state and triggering symptomatic recovery along with the normalization of plasma hormone concentrations. Moreover, the high efficiency of TFC differentiation and follicle morphogenesis in our system will provide an unprecedented opportunity for future studies to decipher regulatory mechanisms involved in embryonic thyroid development. Translating the methodology on human using hESC and iPSCs could give an important contribution in the understanding of the molecular mechanisms underlying congenital hypothyroidism and thyroid.

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S26.2

Dissecting androgen action: new clues from conditional knockout mice

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In males androgens are primarily made by testicular Leydig cells and act as essential regulators of both fetal masculinization and adult reproductive function. The impact of androgens on gene transcription is largely mediated by the androgen receptor (AR), a member of the steroid hormone super-family of ligand activated transcription factors. AR is expressed widely throughout the body, including several key somatic cell-types in the testis. Although we have known for many years that androgens are important regulators of testicular development and function, until recently it has been impossible to determine the specific roles androgens play in each cell-type, and how these cells respond to androgens to ensure correct male development and fertility.
We have exploited conditional gene-targeting of AR using the Cre/lox system to ablate AR function in several key cell-types of the testis, with a view to elucidating the cell-specific roles of androgen-signaling within the testis. These studies have identified novel roles for each cell-type in the promotion of male reproductive function. AR-signaling in SCs controls post-meiotic germ cell development and LC number. AR-signaling in PTM cells controls all stages of GC development, SC function and LC differentiation. Recent unpublished data suggests AR-signaling in LCs is also important for testicular function, acting via a novel mechanism.
Taken together, these studies provide increasing evidence for the presence of a complex androgen-dependent paracrine signaling pathway within the testis, with each AR-expressing cell-type influencing others to ensure their correct development and function.

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S26.3

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Steroids in obesity and metabolism

S27.1

11β-Hydroxystreroid dehydrogenase activity and obesity

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The global epidemic of obesity and type 2 diabetes has hastened the need to identify novel and efficacious therapies. Based upon parallels with Cushing's syndrome, tissue specific cortisol excess, independent of circulating levels, has been suggested to have a crucial pathological role and may represent a potential treatment target. In key metabolic target tissues (liver, adipose and muscle), the endoluminal enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyses glucocorticoid activation of cortisone to cortisol. Rodent models and translation clinical studies have validated its potential as a therapeutic target and pharmacological and genetic manipulation in rodents have show significant metabolic benefits. Selective 11β-HSD1 inhibitors for use in clinical studies have now been developed and clinical data are emerging. Whilst these compounds appear to improve glycaemic control in patients with diabetes and cause improvements in blood pressure and decrease weight some concerns still remain. The magnitude of the clinical benefit that they confer remains to be fully determined and in most studies they cause activation of the hypothalamo–pituitary–adrenal axis. In addition, they have the potential to limit the endogenous anti-inflammatory actions of glucocorticoids, although clinical data to support this are lacking. Whilst their development has focussed on treating metabolic disease, alternative indications are emerging and further clinical studies are now warranted.

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S27.2

Metabolic control through glucocorticoid hormones

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In the past decades, glucocorticoid (GC) hormones and their cognate, intracellular receptor, the glucocorticoid receptor (GR), have been well established as critical checkpoints in mammalian energy homeostasis. Whereas many aspects in healthy nutrient metabolism require physiological levels and/or action of GC, aberrant GC/GR signalling has been linked to severe metabolic disorders, including obesity, insulin resistance and type 2 diabetes. Consequently, molecular mechanisms within the GC signalling axis have become a major focus in biomedical research, up-to-date particularly focusing on systemic glucose and lipid handling. However, with the availability of novel high throughput technologies and more sophisticated metabolic phenotyping capabilities, as-yet non-appreciated, metabolic functions of GC have been recently discovered, including regulatory roles of the GC/GR axis in protein and bile acid homeostasis as well as metabolic inter-organ communication.
This talk will discuss recent advances in GC/GR-controlled energy homeostasis, and summarises findings relevant for basic and translational metabolic research.

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S27.3

Glucocorticoid-mediated fetal programming in humans

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There is convincing evidence in animals suggesting a role for prenatal glucocorticoid overexposure in the programming of adult metabolic diseases. Evidence for glucocorticoid-mediated fetal programming in humans often comes from observational studies in preterm babies with variable disease courses, in whom it is difficult to distuinguish programming from other effects.
Preterm infants early in their neonatal course are subject to stressful and sometimes even critical events, including respiratory distress, intubation and mechanical ventilation, septicaemia, necrotizing enterocolitis, and insertion of venous lines. Obviously, during this period their chances for survival are strongly dependent on the effects of antenatal and/or postnatal glucocorticoid treatment.
However, recent studies have shown that once the neonatal threats are resolved, preterm survivors continue to show effects of increased glucocorticoid bioactivity, such as abdominal fat accumulation, glucose intolerance, and raised blood pressure. Several mechanisms with a central role for hypothalamus–pituitary–adrenal axis functioning may underpin these observations. First, subjects who are genetically more sensitive to the effects of endogenous or exogenous glucocorticoids may survive preterm birth easier but are predisposed later to metabolic diseases. Second, extremely stressful insults in early life may induce site-specific alterations in the epigenetic code of the glucocorticoid receptor gene promotor, leading to decreased central feedback suppression, and thus, enhanced stress responsivenss and disease. Third, exposure to glucorticoids may impact on the development of several organs, depending on the glucocorticoid receptor genotype.

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Autoimmune endocrine disease - Old and new players

S28.1

Autoimmune thyroid disease

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Graves' hyperthyroidism (GH) and Hashimoto's hypothyroidism (HH) are the opposite ends of the clinical expression of autoimmune thyroid diseases (AITD). AITD are multifactorial 'complex' diseases in which immune responses against thyroid antigens develop in genetic susceptible subjects, provoked by environmental factors. Susceptibility genes include immunoregulatory genes (HLA, CTLA-4, PTPN22, CD4 and FCRL3) and thyroid-specific genes (TSHR and Tg). Environmental factors include ambient iodine intake, stress, smoking, particular drugs (interferon, alemtuzumab and HAART), and possibly infections. Endogenous factors (female gender, parity and X-chromosome inactivation) contribute to the risk of AITD. We will focus on new data on the role of environmental factors.
Smoking is a well-established risk factor for GH and especially for Graves' ophthalmopathy. Recent studies provide convincing evidence that smoking to a certain extent protects against HH. Cessation of smoking is associated with *de novo* development of TPO-Ab, and with a transient increase in the incidence of overt hypothyroidism. The remarkable divergent effects of smoking on GH and HH remain incompletely understood.
Alcohol consumption is associated with a decreased risk of *de novo* development of TPO-Ab. It also protects against the development of overt GH or HH, independent of smoking behaviour.
Selenium deficiency might be involved in the pathogenesis of AITD, but the evidence is less clear. Selenium supplementation in Hashimoto's thyroiditis decreases serum TPO-Ab in some but not all studies. Selenium concentrations in GH patients are highest in patients who go into remission after a course of antithyroid drugs.
Vitamin D deficiency. In a prospective study the *de novo* development of TPO-Ab was not associated with low serum 25(OH)D. In view of paracrine effects of locally synthesized $1.25(OH)_2D$ and polymorphisms in the vitamin D receptor it is, however, premature to dismiss a role of vitamin D in the pathogenesis of AITD.

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S28.2

Autoimmune Addison's disease: new players in diagnosis and treatment

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Addison's disease is a rare disorder and mostly due to an immune-mediated adrenal destruction similar to the organ specific loss of function in autoimmune hypothyroidism in Hashimoto's thyroiditis or type 1 diabetes. Adrenal insufficiency occurs thereby as isolated disease or is accompanied by other autoimmune disorders forming the autoimmune polyendocrine syndrome (mostly APS2). A major problem of Addison's disease is that the diagnosis is often late or may be missed when patients present with acute adrenal crisis. A delay in diagnosis may also impair later quality of life despite optimal steroid substitution. A timely diagnosis is mandatory and therefore adrenocortical function testing should be performed in all patients with suspected Addison's disease. Patients at risk are those with other autoimmune disorders, in particular women with primary ovarian failure, any with vitiligo or the relatives of patients with Addison's disease, although the absolute risk is small. Antibodies against the 21-hydroxylase are found in up to 85% of newly diagnosed patients and can be detected prior to manifestation. Such antibody positive individuals must be carefully monitored and – as soon as they become adrenal insufficient- supplemented with hydrocortisone and mineralocorticoids without delay. Although the corticosteroid supplementation pharmacodynamics is a poor mirror of the normal cortisol profile most patients manage their chronic disease in a well adapted manner. There is a subgroup of patients with inadequate results of substitution, a tendency to fall into adrenal crisis and poor quality of life. New modifications of hydrocortisone release may offer an improvement but patients need to be investigated for undetected comorbidities. Vitamin D deficiency is frequently found in patients with Addison's disease and should be corrected.

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S28.3

Hypophysitis

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Autoimmune hypophysitis (AH) is characterized by lymphocytic infiltration and eventually destruction of the pituitary tissue. AH accounts for ~1% of pituitary masses and 0.5% of hypopituitarism. Although it has been recognized as an uncommon pituitary disease, its true frequency is not known but certainly underestimated. AH may affect pre- and post-menopausal women, men and children, but most cases are reported in women during reproductive age. AH may be associated with other autoimmune disorders including Hashimoto's thyroiditis, pernicious anemia, type 1 diabetes mellitus, Graves' disease, autoimmune

adrenalitis. Based on the anatomical location, several types of AH is described as follows: lymphocytic adenohypophysitis (LAH), in which inflammation is limited to the anterior pituitary and it usually manifests during pregnancy, lymphocytic infundibuloneurohypophysitis (LINH), in which male and female are equally affected, and lymphocytic panhypophysitis (LPH). Diabetes insipidus may be seen especially in patients with LINH. Diffuse thickening of the pituitary stalk, enlarged pituitary gland associated with homogeneous contrast enhancement, suprasellar expansion especially tongue-like extension and loss of neurohypophyseal 'bright spot' in the imaging studies are helpful radiological findings in the diagnosis of AH. In spite of normal MRI findings, varying degrees of hypopituitarism may exist in patients with AH. Recent data suggest that AH may be associated with empty sella which may be the final outcome of AH. Empty sella is a common radiological feature of Sheehan's syndrome, for this reason it should be included in the differential diagnosis of AH. The diagnosis of LH is difficult at least in patients without histopathological findings. Presence of antipituitary antibodies (APA) especially antibodies to pituitary hormone-producing cells may be helpful in the diagnosis of AH. On the other hand, recent data have shown that pituitary autoimmunity may play some role in the development of hypopituitarism seen in patients suffered from traumatic brain injury (TBI). Pituitary dysfunction development ratio in TBI has been found to be significantly higher in APA positive patients when compared with APA negative patients.

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Management of thyroid nodules

S29.1

Molecular analysis of FNAB material

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The evaluation of a thyroid nodule is a very common clinical problem and fine needle aspiration biopsy (FNAB) is the only test that can provide a definitive preoperative diagnosis of malignancy. The sensitivity and specificity of FNAB are limited by aspirates that yield insufficient material for definitive diagnosis and those with indeterminate diagnoses, which can account for up to 30–40% of all specimens. The detection of several novel gene mutations in differentiated thyroid cancer (DTC) over the last decade has led to the diagnostic use of these oncogenic alterations to improve FNAB sensitivity and specificity. In recent years, several prospective and retrospective studies have shown that molecular testing of thyroid nodules for a panel of mutations can be effectively performed in a clinical setting and can be useful to improve the diagnosis of malignancy when used as an adjunct to traditional cytology. In particular, either BRaf or ret/PTC, or PAX8/PPARg or ras, alone or as a panel of oncogenes, have been analyzed in FNA specimens by different Authors. Due to the reported association between BRaf and poorer prognosis, the diagnosis of a BRaf positive nodule can help both to diagnose and to identify those PTC patients who may need more aggressive surgical treatment and vigilant clinical monitoring. Cost/benefit studies showed that molecular testing of indeterminate FNAB results is cost saving predominantly because of reduction in two-stage thyroidectomy and can potentially avoid almost three fourths of currently performed surgeries in patients with benign nodules. In conclusion, the clinical application of molecular techniques to detect mutations in thyroid FNAB samples has been shown to improve the preoperative diagnosis for DTCs and is particularly useful for those tumors that are 'indeterminate' by traditional cytological analysis.

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S29.2

Diagnostic pitfalls in fine needle aspiration of thyroid nodules

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Fine needle aspiration (FNA) of thyroid can be confidently enlisted as a primary diagnostic tool in evaluating thyroid nodules to spot cancer, although this confidence should be tempered with awareness of possible diagnostic pitfalls. The sources of pitfalls (toward false positive or false negative results) may lie in sampling, in microscopic interpretation, or even in the clinical evaluation. Nonthyroidal lesions (parathyroid, lymph nodes, and salivary glands) aspirated 'by mistake' as thyroid nodules may lead to false diagnoses. Parathyroidal adenomas are mostly misdiagnosed as thyroid neoplasms. Sampling in the peritumoral tissue with thyroiditis may miss the neoplasm and lead to a false negative diagnosis. Hashimoto's disease is a major source for false positive diagnoses based on the sometimes severe reactive epithelial atypia or on the extensive oncocytic metaplasia mimicking oncocytic neoplasm or papillary Ca (PTC). Conversely, neoplastic cells can be obscured by dense lymphoplasmocytic infiltrates. Metaplastic oxyphilic cells in hyperplastic nodules can also be overdiagnosed as neoplastic ones, whereas oxyphilic cells of PTC are sometimes interpreted as metaplastic. False negative diagnoses of PTC are sometimes due to the cystic degeneration of the neoplasm and the absence of well preserved neoplastic cells in the smears. Degenerative cell changes may be confusing. The neoplastic nature of highly differentiated PTCs, eventually of the macrofollicular, colloid rich variant, may be overlooked. Since the cells of medullary Ca may be small with minor atypia, the neoplasm may be underdiagnosed, especially in a colloid rich background due to admixtures. Sometimes the neoplasm is diagnosed as a lymphoma or a follicular lesion if the specific features are overlooked. A monomorphous pattern of small cells of a poorly differentiated Ca can also be misleading. Paucicellular anaplastic Ca may be missed. Finally, needle tract effects on repeat FNA comprise alterations like reactive follicular or endothelial cells, leading to false positive results.

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S29.3

Follow-up of benign nodules

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The majority of thyroid nodules do not need treatment after the exclusion of malignancy or abnormal thyroid function due to the usually slow growth rate of benign thyroid lesions. Clinical and US follow-up should be performed every 1–2 years. A repeated US-FNA is appropriate only in case of relevant nodule growth or suspicious US changes. A minority of benign nodules present a progressive growth that causes local pressure symptoms or patient's concern. To avoid unnecessary surgery, treatment options include percutaneous ethanol injection (PEI) for recurrent cystic lesions and TSH suppression with levothyroxine (T_4) or US-guided thermal ablation for solid nodules. A clinically significant decrease in nodule volume is obtained with T_4 only in a minority of patients. Routine use of T_4 therapy is not recommended but may be considered, with iodine supplementation, in younger patients from iodine-deficient areas who have small nodules with colloid features on cytology, or small size nodular goiters with no evidence of functional autonomy. If T_4 therapy is not indicated or the nodule grows during the course of treatment, surgery or image-guided mini-invasive procedures may be considered as therapeutic options. PEI is an alternative to surgery for complex nodules with a dominant fluid component. PEI is significantly superior to aspiration alone in reducing nodule volume with a volume decrease $>50\%$ in nearly 90% of cases. For solid benign nodules that cause local symptoms or patient's concern, percutaneous thermal ablation with laser or radiofrequency may be considered as an effective debulking technique. These outpatient procedures are rapid and do not require high-cost devices. US and US-FNA have sharply decreased the rate of diagnostic surgery. US follow-up makes possible an easy and reliable monitoring of benign thyroid nodules. Mini-invasive procedures may further reduce the number of surgical treatments for symptomatic benign nodules.

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Energy Status and pituitary function

S30.1

Energy status and puberty: novel neuroendocrine regulatory mechanisms

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Reproduction is an energy-demanding function. Accordingly, acquisition of reproductive capacity at puberty is metabolically gated, as a means to prevent fertility in conditions of energy insufficiency. In addition, obesity has been shown to impact the timing of puberty and may be among the causes for the earlier trends of pubertal age reported in various countries, especially in girls but probably also in boys. The metabolic control of puberty in such a spectrum of situations, ranging

from energy deficit to extreme overweight, is the result of the concerted action of different peripheral hormones and central transmitters that allow the sensing of the metabolic state of the organism and transmit this information to the various elements of the reproductive brain, mainly the GnRH neurons. In this presentation, we will provide a synoptic overview of recent developments that have deepened our understanding of the neuroendocrine and molecular basis for the metabolic control of puberty onset. These include not only the demonstration of the involvement of the hypothalamic Kiss1 system in the control of puberty and its modulation by metabolic cues, but also the identification of the roles of additional transmitters, such as neurokinin-B and nesfatin-1, and hypothalamic pathways, such as those originating from the ventral pre-mammilary nucleus, in the metabolic regulation of puberty. In addition, recent progress in the identification of putative molecular mediators for the metabolic gating of puberty will be reviewed here. All in all, characterization of these novel players and regulatory mechanisms will improve our understanding of the basis of normal puberty, and its eventual alterations in conditions of metabolic stress, ranging from anorexia to morbid obesity.

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S30.2

Energy status and glucocorticoid excess

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Glucocorticoids (GC) act at different target tissues by binding two different intracellular receptors, the glucocorticoid receptor (GR) and the mineralcorticoid receptor (MR), but the their effects vary considerably between subjects, due to a different sensitivity, which is at least partially, genetically determined. GCs are so named based on their actions on carbohydrate metabolism, namely on insulin-dependent processes. In particular, the effect of GC includes increased hepatic glucose production, decreased insulin-dependent glucose uptake into peripheral tissues, breakdown of muscle and fat to provide additional substrates for glucose production, and inhibition of insulin release from pancreatic β cells. On the other hand, they also deeply influence lipid and protein metabolism, thus modifying body composition. In fact, GC excess is characterized by a series of metabolic complications, including abdominal obesity, systemic arterial hypertension, impairment of glucose tolerance, dyslipidemia, and thrombotic diatesis, which increase cardiovascular risk and mortality rate. Moreover, the detrimental effects of GC on bone mass and turnover as well as the alterations at muscle level, inducing the steroid-mediated myopathy, are known as well. Although there is convincing evidence in the literature that clear hypercortisolemia is associated with several metabolic alterations, it is matter of debate whether subclinical GC excess is responsible for the above mentioned abnormalities. Moreover, the remission GC excess usually reduces but does not eliminate these systemic complications and does not completely normalize body composition, thus indicating that the detrimental effects of GC probably last for very long time and that an early diagnosis and cure may, possibly, prevent a negative prognosis of these patients.

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S30.3

Energy status and GH/IGF1 axis

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GH levels decline with age and weight gain and are enhanced in response to nutrient deprivation. Under these circumstances, GH levels are negatively correlated with IGF1 and insulin levels. Since IGF1 and insulin can directly inhibit GH synthesis and release from primary pituitary cells of different species, it is commonly accepted that changes in circulating IGF1 and insulin serve to directly regulate somatotrope function in response to metabolic extremes. However, teasing apart the relative contribution of IGF1 and insulin has been hampered by the fact that: i) the receptors for both hormones (IGFIR and INSR) are structurally/functionally related (i.e. high insulin levels can activate IGFIR), ii) INSR and IGFIR are expressed in the pituitary at comparably high levels; and iii) INSR and IGFIR can interact by forming hybrid receptors or via crosstalk of downstream intracellular signaling pathways, thereby modifying the response to their respective ligands. In order to evaluate the separate roles of insulin and IGF1 in regulating somatotrope function, we have used the Cre/loxP system to inactivate INSR or/and IGFIR in the somatotropes, *in vitro* and *in vivo*. Several novel concepts have emerged from this series of studies. First, although insulin and IGF1 ultimately inhibit GH synthesis/secretion, the *in vitro* and *in vivo* mechanisms of action are distinct. Second, somatotrope-specific loss of INSR or/and IGFIR increases GH levels, which cannot be completely compensated for by an intact hypothalamic feedback system, thereby establishing the somatotrope as a primary sensor for changes in circulating insulin/IGF1 levels. Third, under lean conditions, somatotrope-specific loss of INSR has a more profound effect on circulating GH/IGF1 than loss of IGFIR, suggesting insulin plays a primary role in regulating short-term changes in GH secretion. Finally, in the context of diet-induced obesity, the direct effects of insulin (or IGF1) on somatotrope function cannot fully account for the fall in GH levels and therefore, must be driven by insulin-induced changes in hypothalamic function or other systemic factors that act centrally or directly on the somatotrope to reduce GH output.

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Clinical impact of rare mutations in endocrinology

S31.1

Does a new mutation always predict a new disease? Lessons from p27 mutations

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A few years ago a novel multiple endocrine neoplasia syndrome, named MEN type 4 (MEN4), was discovered thanks to studies conducted on a MEN syndrome in the rat (named MENX). Rat and human syndromes are both caused by germline mutations in the *Cdkn1b*/*CDKN1B* gene encoding p27Kip1, a putative tumor suppressor which binds to and inhibits cyclin/cyclin dependent kinase complexes, thereby inhibiting cell cycle progression. MEN4 patients carry heterozygous mutations at various residues of p27Kip1 and present with endocrine lesions mainly belonging to a MEN1-like spectrum. Indeed, the most common phenotypic features in mutation carriers are parathyroid and pituitary adenomas. Recently, germline mutations in p27kip1 were also identified in patients with a sporadic presentation of parathyroid adenomas. *In vitro* functional characterization of several *CDKN1B* sequence variants identified in MEN4 patients, detected impaired activity of the encoded p27Kip1 mutant proteins (e.g. reduced expression, cytoplasmic mislocalization or poor binding to interaction partners). These results indirectly pinpoint the characteristics of the p27Kip1 protein which are critical for tumor suppression. Although the number of MEN4 patients is still very low, the discovery of this syndrome has demonstrated a novel role for *CDKN1B* as a tumor susceptibility gene for neuroendocrine tumors. In this lecture, I will review clinical and molecular characteristics of the MEN4 syndrome and the role of p27Kip1 in neuroendocrine tumorigenesis.

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S31.2

Old and new MEN1 mutations

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Multiple endocrine neoplasia type 1 (MEN1 – OMIM 131100) is considered as the major hereditary syndrome predisposing to multifocal endocrine tumors and a broad spectrum of non-endocrine lesions. Since the cloning of the MEN1 gene in 1997, a large number of studies have shown the large diversity of germline mutations found in patients with a poor genotype – phenotype correlation. Menin, the protein encoded by the MEN1 gene interacts with more than 30 nuclear/cellular factors (menin interacting proteins or MIP's) involved in major processes of cell life. Among them, JunD, a negative regulator of the AP1 transcription factor, and mSin3A, a cofactor of chromatin remodeling complexes, are critical determinants of the functional pathway involving menin in the negative regulation of cell proliferation. A powerful network, called Groupe d'étude des Tumeurs Endocrines (GTE) has been initiated in France in 1995 and represents today a comprehensive database on MEN1 patients identified through

clinical centers in the whole country. In a recent study on a cohort of 806 patients from a total of 262 families, we reported that the overall risk of death directly related to MEN1 tumors was significantly higher when mutations affected the JunD interacting domain. Such a correlation should thus be considered for surgical indications, genetic counseling and clinical follow-up. From these first epidemiological studies, we focus on the putative data provided by a large cohort studies, in relation to the functional areas of interaction with the MIP's. Preliminary data are also presented on a number of missense variants which have been initially considered as polymorphisms, and shown to disrupt functional interactions of menin. These studies show that specific genotype phenotype correlations, unidentifiable with the 'old' series of mutations, may now be characterized by working on very large cohorts of patients for which complete clinical and genetic information have been collected.

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S31.3

Genetic diagnosis of hereditary neuroendocrine syndrome in asyntomatic patients: clinical and prognostic implications

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Neuroendocrine tumours (NETs) can be sporadic or can arise in complex hereditary endocrine disorders such as multiple endocrine neoplasias (MENs), familial paragangliomatosis (FPGLs), neurofibromatosis type 1 (NF1), von Hippel–Lindau disease (VHL), tuberous sclerosis (TSC). It has been estimated that hereditary NET occurrence varies with site of origin of the tumour, representing 5–30% of all cases of NET. These rates seems to be an underestimation and novel mutations of well known oncogenes or tumour suppressor genes as well as new genes and molecular pathways responsible for unknown syndromes are expected to be characterized.

Hereditary NETs generally occur at an earlier age and show higher secretive activity than sporadic ones. Diagnosis is made around sixth decade of life in sporadic NETs while it is anticipated of about three decades in hereditary tumours. The identification of hereditary NET syndromes is relevant to achieve a precocious diagnosis and this may be important to prevent severe complications and unfavourable outcome. For this reason, the genetic screening is nowadays a well established procedure in many tumour types allowing to reclassify as carrier of specific hereditary NET syndromes, a number of patients with an apparently sporadic tumours. Some studies, focusing in particular on MEN type 1, highlighted that the genetic screening impacts on the management and clinical outcome of NETs, because it allows to detect tumours at an early stage or even before their development.

In spite of these recent advances, at now, clinical pictures of most of the hereditary NET syndromes are incomplete or not updated. Furthermore, follow-ups of these patients are not standardized.

In summary, the genetic diagnosis has a strong impact on the clinical course and prognosis of hereditary NETs. However, natural history remains to be defined more in deep for most of the actually known hereditary NET syndromes.

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Is diabetes a lipid disease?

S32.1

Abstract unavailable.

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S32.2

Fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) is becoming a master component of the epidemic of obesity and metabolic syndrome worldwide due to excessive caloric intake. The spectrum of NAFLD ranges from simple fatty liver with benign prognosis to a potentially progressive form, nonalcoholic steatohepatitis (NASH), which may lead to liver fibrosis and cirrhosis resulting in increased morbidity and mortality. Development of hepatic steatosis and its progression to steatohepatitis may be the consequence of dysfunction of several metabolic pathways, such as triglyceride synthesis, VLDL secretion and fatty acid β-oxidation. One main determinant in the pathogenesis of fatty liver seems to be an increment in the serum fatty acid pool. The sources of fat contributing to fatty liver are peripheral TGs stored in white adipose tissue that are driven to the liver in form of plasma non-esterified fatty acids (NEFAs), dietary fatty acids, and hepatic de novo lipogenesis (DNL). The metabolic partitioning of fatty acids between mitochondrial β-oxidation and TG synthesis is critically regulated. In the liver, fatty acid β-oxidation is normally inhibited by food intake by the action of insulin, which is the main regulator of DNL due to its direct activation of SREBP1c. Recent studies focused on the transcriptional regulatory proteins that drive mitochondrial biogenesis and oxidative metabolism, lipogenesis and triglyceride (TG) secretion. We will present novel data from tissue-specific mouse models that bolster the concept that a combined non-insulin driven hepatic specific action on lipid synthesis and secretion, as well as, on mitochondrial biogenesis and function could protect against fatty liver and insulin resistance.

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S32.3

Adipose tissue lipolysis and insulin sensitivity

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When energy is needed, white adipose tissue (WAT) provides fatty acids (FA) for use in peripheral tissues via stimulation of fat cell lipolysis. FA have been postulated to play a critical role in the development of obesity-induced insulin resistance, a major risk factor for diabetes and cardiovascular disease. However, whether and how chronic inhibition of fat mobilization from WAT modulates insulin sensitivity remains elusive. Hormone-sensitive lipase (HSL) participates in the breakdown of WAT triacylglycerol into FA. HSL haploinsufficiency and treatment with a HSL inhibitor resulted in improvement of insulin tolerance without impact on body weight and fat mass in high fat diet-fed mice. Notably, WAT inflammation was not modified. *In vivo* palmitate turnover analysis revealed that blunted lipolytic capacity is associated with diminution in FA uptake and storage in peripheral tissues of obese HSL haploinsufficient mice. The reduction in FA turnover was accompanied by an improvement of glucose metabolism with a shift in respiratory quotient, increase of glucose uptake in WAT and skeletal muscle and, enhancement of *de novo* lipogenesis and insulin signalling in liver. In human adipocytes, HSL gene silencing led to improved insulin-stimulated glucose uptake resulting in increased *de novo* lipogenesis and activation of cognate gene expression. In clinical studies, WAT lipolytic rate was positively and negatively correlated with indexes of insulin resistance and WAT *de novo* lipogenesis gene expression, respectively. In obese individuals, chronic inhibition of lipolysis resulted in induction of WAT *de novo* lipogenesis gene expression. Thus, reduction in WAT lipolysis reshapes FA fluxes without increase of fat mass and improves glucose metabolism through cell-autonomous induction of fat cell *de novo* lipogenesis which contributes to improved insulin sensitivity.

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Meet the Expert Sessions

MTE1

What systems biology can do for endocrine research?

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Systems biology views and studies the biological systems in the context of complex interactions between their building blocks and processes. Given its multi-level complexity, metabolic syndrome (MetS) makes a strong case for adopting the systems biology approach. Despite many MetS traits being highly heritable, it is becoming evident that the genetic contribution to these traits is mediated via gene–gene and gene– environment interactions across several spatial and temporal scales, and that some of these traits such as lipotoxicity may even be a product of long-term dynamic changes of the underlying genetic and molecular networks. This presents several conceptual as well as methodological challenges and may demand a paradigm shift in how we study the undeniably strong genetic component of complex disorders such as MetS. The argument will be made that for adopting systems biology approaches to MetS an integrative framework is needed which glues the biological processes of MetS with specific physiological mechanisms and principles and that lipotoxicity is one such framework. The metabolic phenotypes, molecular and genetic networks can be modeled within the context of such integrative framework and the underlying physiology.

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MTE2

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MTE3

Insulin therapy

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Insulin replacement therapy is essential for anyone with type 1 diabetes. Most patients with gestational or type 2 diabetes may also require insulin. The goals of insulin therapy are: to achieve optimal glycemic control without causing hypoglycemia or excessive weight gain and to minimize the impact on lifestyle. The therapeutic goals should be individualized according to patient's age, disease duration, complications, comorbidities, lifestyle, and expected survival.

Selection of insulin regimens depends on residual endogenous insulin or insulin resistance, glucose control, and daily activities. The later insulin therapy is initiated, the less likely that target HbA1c will be achieved.

Insulin therapy is not without challenges. Patient's and/or physician's resistance to insulin need to be overcome. As a consequence, starting and adhering to therapy has been difficult for both sides. Generally insulin analogs are not superior to human insulin in achieving glycemic control. However, the risk of hypoglycemia is lower, and they provide more flexibility.

There is no right way to initiate insulin therapy but there are several options and some guidelines derived from clinical trials. Insulin regimens for type 1 diabetes patients consist of basal-bolus components. The initial dose can be calculated 0.3–0.5 IU/kg per day given as 50% basal and 50% bolus in divided doses before meals. Carbohydrate counting and correction insulin doses may help to achieve better glycemic control.

The initial dose for basal insulin in patients with type 2 diabetes is usually 0.2–0.3 IU/kg per day. If glycemic control is not achieved with basal insulin alone, intensification of insulin therapy with addition of a regular or rapid-acting insulin prior to meals is indicated. Alternatively, a premixed biphasic formulation can be used before breakfast and dinner.

Insulin pump therapy may provide more flexible daily life with less frequency of hypoglycemia in most patients with type 1 and in some with type 2 diabetes.

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MTE4

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MTE5

Current guidelines for the classification of net

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Neuroendocrine neoplasms (NENs) are a variety of neoplastic lesions distributed in most organs and apparata. The current World Health Organization (WHO 2010) introduced tumor grading (G1–G3) and tumour node metastasis (TNM) staging for class definition. The proposed grading system defines three classes (G1–G3) according to both mitotic count and Ki67 index. Three classes were defined as WHO class 1, neuroendocrine tumor (NET), G1; WHO class 2, NET G2 and WHO class 3, neuroendocrine carcinoma (NEC), by definition G3. The definition of NET equalizes the previous definition of 'carcinoid' (typical and atypical as variably utilized in the pathology literature) and of well-differentiated endocrine tumor/carcinoma as from the previous WHO 2000 classification. The WHO 2010 TNM substantially endorsed the classification originally proposed by the European Neuroendocrine Tumour Society (ENETS) with two exceptions in that i) it limits its application to NET G1–G2 (carcinoids) and ii) it shows significant differences for pancreas and appendix TNM definitions. In general a common staging system frame was defined with stage I tumors with limited growth, stage II larger or more invasive tumors, in absence of metastases, stage III tumors invading the surrounding structures or with loco-regional metastases and stage IV implying distant metastases. This classification system was first adopted by the International Union Against Cancer (UICC) and subsequently endorsed by both the American joint Cancer Committee (AJCC) and the WHO. Current oncology guidelines for Neuroendocrine neoplasms are largely based on proliferation fraction and stage to define specific therapeutic options.

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MTE6

New immunotherapy approaches for Graves' orbitopathy

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The role of B-cell in human autoimmune disease have recently been emphasized due to the therapeutic benefit of B-cell depleting therapies. B cells are involved in the production of autoantibodies, CD4 + T-cell activation and control of T-cell function and inflammation, through cytokine production. Although autoantibodies alone may not initiate autoimmune disease, their relationship with the disease course suggests that they are a key factor contributing to the mechanisms of disease pathogenesis. B cells are also important antigen presenting cells? Rituximab (RTX) has been used off-label in various autoimmune disorders and effectively depletes mature and memory CD20 + B cells, but not long-lived plasma cells. This has provided the rationale for the use in Graves' disease (GD), since blockade of pathogenic autoantibody generation might induce Graves' hyperthyroidism remission. Although we suggest caution in proposing RTX as a novel therapeutic tool in this disease, preliminary data collected by us and others show that RTX does significantly affect the inflammatory activity and severity of GO. Recently, we have reported that low dose of RTX (100 mg), about 20-fold less than the commonly administered dose, caused effective peripheral B cell depletion and induced long term remission of GO, without further treatment. The amelioration of GO has not been different from what has been reported with larger doses of RTX. This study is potentially interesting also from the point of view of the safety concerns when using higher doses of a potent immunosuppressive agent like RTX. We envisage that the optimal strategy for controlling the progression of a disease like GO would be to pursue B-cell depletion shortly after diagnosis and not solely as additional therapy when standard immunosuppression has failed.

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MTE7

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MTE8

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MTE9

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MTE10

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MTE11

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MTE12

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MTE13

Hypogonadotropic hypogonadism

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Patients with congenital hypogonadotropic hypogonadism (cHH; also referred to as idiopathic HH) have inadequately low gonadotropin and sex steroid levels for age without an underlying organic or functional cause. The combination of cHH and deficient sense of smell is called Kallmann syndrome (KS; incidence 1:30 000 in Finnish boys, and 1:125 000 in girls), which is due to misrouting of primitive GnRH neurons *in utero* from the olfactory placodal area to the developing hypothalamus.

The phenotypic features of cHH are variable. Hallmarks of severe reproductive phenotype include micropenis and/or cryptorchidism, whereas those with a partial reproductive phenotype may show some signs of gonadotropin and sex steroid action. Examples of associated phenotypes include cleft lip/palate, hearing loss, synkinesia, and limb anomalies. Approximately 10% of male cHH patients display reversal of hypogonadotropism later in life after androgen exposure.

Patients with cHH may have family members with cHH, delayed puberty, cleft lip/palate, infertility, cryptorchidism, or hyposmia/anosmia. In clinical examination, special attention should be paid on the stage of puberty, size of the testes, and on the presence of associated phenotypes. Chronic diseases, multiple pituitary hormone deficiency, syndromes, and organic and functional causes of HH should be ruled out. In addition to measurement of gonadotropin and sex steroid levels, Sertoli cell-derived inhibin B and AMH levels together with GnRH and hCG stimulation tests are usually of diagnostic value.

All patients with cHH should be referred to genetic counseling. Currently, 30-40% of cHH patients can be given a molecular genetic diagnosis. Certain phenotypic cues help to prioritize mutation screening. For example, semicircular canal hypoplasia should prompt testing of the *CHD7* gene, and patients with anosmia in combination with bimanual synkinesia should be first screened for a mutation in *KAL1*. Biallelic defects in *GNRHR*, *GNRH1*, *TAC3*, *TACR3*, *KISS1*, *KISS1R* underlie normosmic cHH, and mutations in *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, or *CHD7* have been reported in cHH patients with normal or deficient sense of smell.

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MTE14

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MTE15

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MTE16

Diabetic foot disease for endocrinologists

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Foot complications are common in people with diabetes. As the incidence of diabetes continues to rise and an increasingly aged population accrue substantial numbers of non-reversible, chronic disease processes, the burden of diabetic foot disease can only grow. Ulceration and amputation are associated with high mortality, reduced quality of life and significant financial cost to both patient and health care provider.

The management of diabetic foot disease is not the preserve of a single specialism, surgical or medical. Rather, there is an absolute requirement for a multi-faceted team to attend to the myriad of pathological processes which are increasingly seen in affected patients.

By its very nature endocrinology requires an understanding of how disorders in one system can have ramifications in sites some way distant. Endocrinologists are trained to assess pathophysiology in an integrated and holistic manner, are well

used to working in multidisciplinary teams in other aspects of their practice and are therefore well placed to take on an important role at the heart of a service addressing the needs of a fragile patient group.

The heterogeneity of the patient population makes definitive guidelines a challenge and the lack of a robust evidence base for interventions can engender a lukewarm response in the face of a pressing clinical need. Using 'real world' clinical examples, this session will cover the common clinical presentations of acute foot disease and consider how to utilise the skills and input from all members of the clinical team in tackling and successfully managing this oft neglected area to good effect.

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JOE/JME Prize Presentation Sponsored by *Journal of Endocrinology*

Enhancing radioiodine uptake in thyroid cancer

JP1

Enhancing radioiodine uptake in thyroid cancer

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Ablative radioiodine therapy is critical to the treatment of differentiated thyroid cancers and their metastases, and relies on the innate ability of thyroid cells to uptake iodide via the sodium iodide symporter (NIS). As tumours with reduced avidity for radioiodine have a poorer prognosis,it is critical to identify ways to induce NIS activity and hence radioiodine uptake.

Pituitary tumor transforming gene (PTTG) and its interacting partner, PTTG-binding factor (PBF), areproto-oncogenes that are significantly upregulated in thyroid tumours and have been identified as potent repressors of iodide uptake. Higher levels of PTTG and PBF expression are independently associated with early tumour recurrence and an overall poorer disease outcome. Both PTTG and PBF can inhibit NIS transcriptional regulation via an upstream enhancerelement (hNUE). NIS can also be post-translationally regulated through an interaction with PBF that internalises it away from its functional localisation at the cell surface. PBF elicits a similar mechanism of post-translational regulation on the monocarboxylate transporter 8 (MCT8), which mediates the secretion of thyroid hormone, suggesting a role for PBF in the overall regulation of thyroid hormone biosynthesis and secretion.

Recent studies have focused on inhibiting NIS repression by PBF. PBF has been identified as a phospho-protein that can bind the proto-oncogene tyrosine-protein kinase Src. Src significantly induces phosphorylation of PBF residue Y174 and, conversely, Src inhibition reduces Y174 phosphorylation. Of direct clinical importance to the treatment of thyroid cancer, Src inhibition entirely overcomes PBF repression of iodide uptake in human primary thyroid cells. Hence, targeting PBF phosphorylation at residue Y174 via tyrosine kinase inhibitors may be a novel therapeutic strategy to enhance the efficacy of ablative radioiodine treatment in thyroid tumours.

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Endocrine Nurse Symposium

EN1.1

Pre- and post-operative care of the patient undergoing pituitary surgery

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The care of the patient undergoing pituitary surgery is complex and requires detailed knowledge of the underlying diagnosis of the patient. Although the care of the patient is similar with each type of hormonally active tumour, specific knowledge is paramount for a successful post-operative outcome. In this presentation, the medical and surgical management of common pituitary tumours will be discussed. Patients with Cushing's disease and acromegaly will be highlighted. The pre- and post-operative nursing assessment, surgical resection of tumour, and post-operative management, including common complications will be discussed. At the end of the lecture, the endocrine nurse will have a working knowledge of the care of the patient undergoing pituitary surgery.

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EN1.2

Pituitary dynamic testing

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Due to the pulsatile characteristic of pituitary hormone secretion, its involvement in the acute response to stress and feed-back mechanisms with hormones of peripheral glands, baseline circulating levels of many pituitary hormones may significantly overlap between normal subjects and patients with pituitary disease. Therefore pituitary testing has been widely used for the diagnosis and follow-up of pituitary disease.

For example, provocative tests are frequently used in the diagnosis of adult GH deficiency (GHD), because normal IGF1 levels do not rule out severe GHD. The insulin tolerance test (ITT) is still considered as the test of reference but it should be recognized that other, less bothersome, tests are as reliable as the ITT, for example the GHRH/arginine stimulation test.

The endocrine nurse has an important role in conducting these tests. This role involves more than just administrating the diagnostic agent and blood sampling. Firstly, the endocrine nurse has a job in providing information to the patient about the purpose, adverse effects, and practical execution of the test. Secondly, it is very important that the endocrine nurse observes the patient well and that he/she is able to act quickly and correctly upon complications or problems. The problems that the endocrine nurse may face are divers and require an adequate approach, without affecting the test. Any problems should be clearly communicated with the treating endocrinologist. Thirdly, the endocrine nurse should guide the patient during this whole process. Finally, the endocrine nurse has a role in identifying and solving structural problems. In addition the nurse can take responsibility in writing and updating protocols in collaboration with an endocrinologist. All of this demands knowledge of anatomy and physiology of the endocrine system, knowledge about the various function tests and knowledge about blood sampling techniques and laboratory techniques.

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EN1.3

Abstract unavailable.

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EN1.4

GH replacement in adults

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GH deficiency in adults is associated with physical, psychological and quality of life impairment. A vast range of treatment options is available for GH and with the right device and adequate education and monitoring, patients report a significant improvement in their quality of life. A holistic approach should be adopted when deciding on a treatment regime for GH, which takes into consideration the patient's physical/cognitive ability and their social environment. The Endocrine Nurse has a vital role in selecting the right treatment and monitoring patient's progress. Biochemical parameters and quality of life questionnaires provide useful information, however, they should be combined with regular consultations, continuing education, and patient's active involvement in their treatment to provide the best outcome.

At University College Hospital in London we have a caseload of 250 patients on GH. Following diagnosis of GH deficiency, patients are referred to the Endocrine Nurse for treatment initiation and long term monitoring based on an evidence based protocol. A detailed face to face initial consultation ensures that patients receive the right education and information about their treatment and long-term monitoring. Patients are then transferred to a Nurse-led telephone clinic for six monthly follow ups, although these do not replace their routine face to face pituitary clinic appointments. A recent audit showed that patients on GH monitored in the Nurse-led Clinic report better levels of satisfaction with and adherence to treatment as well as biochemical parameters compared to patients monitored in the community by their General Practitioner. This supports the need for patients on GH to be monitored in a Nurse-led Clinic, although the Endocrine Nurse should be aware of the risk of other team members being de-skilled and should organise regular educational sessions which will provide them with the up to date developments in this area.

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EN1.5

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EN2.1

The role of the endocrine nurse in the care and treatment of children with congenital adrenal hyperplasia and in their transition to the adult service

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Congenital adrenal hyperplasia (CAH) describes a group of autosomal recessive disorders characterized by impaired cortisol biosynthesis. The most common form of CAH is due to 21-hydroxylase deficiency and there is a wide range of phenotypes. The challenge in management of CAH in childhood is promotion of normal growth and development. The morbidities associated with currently available therapeutic regimens are related to glucocorticoid and/or androgen excess, intertwined with the difficulty of achieving a balance between over- or under-treatment. Once linear growth is completed, the management of CAH focuses on coordination of health care and prevention of long-term complications such as obesity, infertility, osteoporosis, and metabolic syndrome.

A multidisciplinary team approach, including endocrinologists (pediatric and adult), endocrine nurse specialists (pediatric and adult), geneticist, urologist, gynecologist, psychologist, social worker, and nutritionist is recommended to optimize the health outcomes of the young adult and their transition to adult service. The endocrine nurse specialist often assumes the role of coordinator in order to facilitate communication between the pediatric and adult teams and the young adult/family to ensure a smooth transition of care, as well as to reduce fragmentation of care. The role of endocrine specialist nurses also includes education and advice to the young adult about achieving independence in the care of their medical condition, which includes planning for emergency care and travel, and moving away from parental home. In addition the role of the nurse involves ongoing assessment of response to changes in treatment, discussion about issues related to sexuality, and education and counseling about risk taking behaviors. Future studies are needed to identify effective intervention models for health promotion in the care of the young adult with CAH that include outcome measures such as quality of life, patient satisfaction, and cost effectiveness.

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EN2.2

Management of CAH in adults

Richard Ross
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CAH is the commonest inborn endocrine disorder and associated with significant morbidity. The health status of CAH adult patients has recently been reported by the UK Congenital adrenal Hyperplasia Adult Study Executive, CaHASE (Arlt *et al*. *JCEM* 2010 **95** 5110–5121). Compared to the health survey for England, metabolic abnormalities were common in adult patients with CAH: obesity (41%), hypercholesterolemia (46%), insulin resistance (29%), osteopenia (40%), and osteoporosis (7%). HR-QOL (SF-36) showed significant impairment similar to scores in heart failure. The CAH patients were taking different glucocorticoid therapies at various doses ($n=196$): hydrocortisone ($n=25$M, 26W), prednisolone ($n=21$M, 67W), dexamethasone ($n=15$M, 22W), or combination therapy ($n=4$M, 16W). The CaHASE group have hypothesised that steroid dose mediates some adverse metabolic outcomes. ANOVA and univariate regression analysis only showed weak correlations ($r<0.2$) between prednisolone equivalent dose and SBP and DBP, HDL-cholesterol and HOMA-IR. However, using principal component analysis (PCA), it was identified that disease control factors, BP and mutation severity are associated with both the choice and total dose of glucocorticoid prescribed. Studies, independent of CaHASE, have examined the development of a modified release formulation of hydrocortisone, Chronocort, for the treatment of adults with CAH. Chronocort, in dexamethasone suppressed normal individuals, is capable of recreating the physiological rise in overnight cortisol levels and in adult patients with CAH improved control of morning 17-hydroxyprogesterone compared to immediate release hydrocortisone. In conclusion, health status in adults with CAH is significantly impaired and Chronocort represents a foundation for future drug development in the pursuit of physiological cortisol replacement.

CaHASE is grateful to the Society for Endocrinology for management of the project and The Clinical Endocrinology Trust for financial support.

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EN2.3

A patient's perspective of CAH: the importance of care

Rick James
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Having been diagnosed with congenital adrenal hyperplasia at the age of 5. I have been lucky enough to turn out as an adult with minimal impact from the condition, and lead a broadly normal life. This brief talk intends to provide a brief diagnostic background for my case, but mainly to focus on the lasting benefits that medical professionals can provide to a patient.

When dealing with children, the importance of providing factual education to the parents is broadly common place, but beyond facts, what about the attitude to a condition? Parents' fears of harm coming to their children, and patients' lack of understanding over consequences to low adherence to medication regimes can undermine excellent treatment at any age. The importance of the support is no less than the importance of medicinal treatment.

This talk is largely subjective, although based on my own experiences. Hopefully it will give some food for thought about how, even though contact time with a patient will often be $<1\%$ of the time in a year, the importance of care can provide a lasting benefit for 100% of the year.

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EN2.4

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EN2.5

Abstract unavailable.

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EN3.1

Endocrinology nursing in Denmark

H. Høi
Denmark.

Introduction

A summary of developments in nursing over the past years in the specially endocrinolodical diseases. The presentation will respectively be in a nursing perspective and in a patient perspective. Furthermore it will be based on nursing for patients with acromegaly, Cushing', Addison' and pheochromocytoma.

The results show a change in the quality of medical care for these groups of patients. It emerged from the nurse's closer contact with patients in the investigation phase and the course of treatment. The nurse's increased knowledge of the patient categories has made this possible.

Patient's involvement and the possibility of close telephone contact has given patients greater peace of mind and quality of life.

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EN3.2

Incorporating research in to the role of the endocrine nurse: an example from my practice

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Incorporating research findings into clinical practice is the primary reason why research is done. The main goal is to offer evidence-based care to the patients. Retaining the nursing findings and integrating them into the healthcare will provide an injustice to the nursing profession.

Obtaining support from the leader team is important in a succesful research nursing project.

We also need resources, time, training and energy if we will create an environment in which nursing research is accepted and necessary. Further, keeping research projects in a short time-frame will help maintain motivation and attention among the patients, as well as the health care actors. Results will be seen faster and satisfaction fulfilled from completing a task and seeing the light at the end of the tunnel.

At the Department of Endocrinology in Lund, Sweden, we have performed a research project in cranially irradiated survivors of childhood acute lymphoblastic leukemia (ALL) with pituitary insufficiency. The survival after childhood ALL has improved dramatically and late complications are common after cranial radiotherapy, with cardiovascular mortality as the leading cause of death. We have shown that 90% of cranially irradiated adult survivors of childhood ALL have GH deficiency, and that GH therapy decreases the risk of cardiovascular complications. Twenty years after diagnosis we also recorded prolactin (PRL) insufficiency and a high prevalence of lactation failure. Further, we have shown a central adrenal insufficiency among 38% of our ALL survivors, and a decrease in bone mineral density *z*-scores, indicating a future risk of osteoporosis. We have implemented the research results into clinical practice at our department and we offer the survivors a multidisciplinary surveillance with specific treatments and recommendations. We are now planning to implement the program at all University Hospitals in Sweden.

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EN3.3

Competency framework for the endocrine clinical nurse specialist

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There is currently no set framework against which an adult endocrine nurse specialist can measure their practice. In December 2010, a working party was formed by the nurse committee of the Society for Endocrinology to draw up a competency framework for Adult Endocrine Nurses. The working party comprised of eight experienced endocrine specialist nurses with a mixture of adult and paediatric experience working in both NHS and private settings. The frame work was developed over a 2-year period.

Nine competencies were formulated, chosen because they are the most common group of conditions cared for in a nursing capacity by Endocrine specialist nurses. These were for acromegaly, Cushing's syndrome, dynamic function testing, GH deficiency, hypogonadism, hypopituitarism, steroid replacement therapy, thyroid, and transitional care of the young adult.

These competencies are divided into three sections: competent, proficient, and expert with each of the levels encompassing the previous level. The working party discussed the skills, knowledge and aptitude required for each topic at each level and how to demonstrate the level of expertise reached and to set targets for future development. We recognised that endocrine nurses may have different levels of expertise for each competency and may not be working at the same level on all of these competencies at a given time. Whilst the document suggests a time scale for development of the nurse from novice to expert it is left to the individual departments to decide.

The framework can be adapted to suit service requirement and can be used for appraisals to set targets and objectives as well as monitoring progress.

This is a first part of an ongoing process where further competencies will be added as the document is used and evaluated.

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EN3.4

Abstract unavailable.

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EN3.5

Abstract unavailable.

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Oral Communications

Pituitary & Molecular Endocrinology

OC1.1 – ESE Young Investigator Award

The consequences of changing endogenous GH/IGF1 levels on carcinogen-induced mammary gland tumorigenesis are dependent on metabolic status in mice

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Introduction
GH and IGF1 are thought to promote breast carcinogenesis as circulating levels of GH/IGF1 are positively correlated with breast cancer risk in epidemiologic studies, and mouse models with developmental GH/IGF1 deficiency or resistance are less susceptible to breast cancer development. However, no studies have shown that high levels of circulating GH/IGF1 can promote mammary tumorigenesis. In this study, two mouse models with elevated or reduced levels of endogenous GH/IGF1 (HiGH and adult-onset GH-deficient (AOiGHD) mice respectively) were used to test the hypothesis that changes in endogenous GH/IGF1 levels can alter the sensitivity of the mammary gland to tumor formation under normal or diet-induced obese conditions.
Methods/design
8–10-week-old chow-fed HiGH, AOiGHD and their respective controls were treated with DMBA (500 mg/10 g BW) for 5 consecutive weeks and the development and progression of mammary gland tumors monitored for 24-weeks. Additionally, HiGH and control mice were fed a high-fat diet for 4-weeks, treated with DMBA and monitored during 20-weeks.
Results
The number of AOiGHD females that developed mammary gland tumors was reduced compared to controls, where tumor size and multiplicity were also reduced. Unexpectedly, mammary tumor formation was not increased in chow-fed HiGH mice. In fact, there was a non-significant reduction in tumor multiplicity and a significant delay in tumor latency. Moreover, the number of mice that develop large tumors (>1 cm) was reduced in HiGH mice. In marked contrast, diet-induced obese HiGH mice presented reduced tumor latency and increased tumor incidence, size and multiplicity.
Conclusion
Under normal metabolic conditions (chow-diet), reduced endogenous GH/IGF1 levels in adults protect against mammary gland tumor formation, while elevated endogenous GH/IGF1 levels do not hasten tumor formation. However, under excessive caloric intake, elevated endogenous GH/IGF1 levels accelerate mammary gland tumor formation and progression, indicating the ultimate consequences of GH/IGF1 on breast tumors development is dependent on the metabolic status.

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OC1.2

Involvement of the constitutive activity of the GHS-R1a (ghrelin G-protein coupled receptor) in the tumorigenesis of somatotroph adenomas

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Pituitary tumors are most usual intracranial tumors, displaying hormonal hypersecretion with in some cases a sustained cell proliferation. The somatotroph adenomas are characterised by a GH hypersecretion. The current treatments are based on somatostatinergic or dopaminergic agonists. Unfortunately, there is steel 50% of patients, which remain insensitive to these treatments. The aim of our work is to find a pharmacological alternative to treat the patients resistant to the current therapies. Ghrelin stimulate pituitary GH release in vivo trough GHS-R1a activation. Interestingly, this receptor not only transduces signal via Ghrelin binding, but also through an unusual high constitutive activity. Noteworthy, human somatotroph adenomas expressed a high level of GHS-R1a at both mRNA and protein level. We actually assess the implication of this constitutive activity in the tumorigenesis of the somatotroph adenomas.
Firstly we demonstrated GHS-R1a functionality through its capacity to fixe endogenous ligand, performing Fluo-ghrelin tracking. Then we showed that treatment of human somatotroph adenomas primary cultures, with the GHS-R1a inverse agonist (modified substance P (MSP)), induced a dose dependent decrease of GH secretion. Apoptotic cell death could also be promoted by MSP treatment.
To foremost investigate the transduction mechanisms underlying these results, we developed, from GH4C1 (rat somato-lactotroph tumoral cell line), stable cell clones overexpressing human GHS-R1a (named MYST-Rg). Interestingly, hGHS-R1a overexpressing cells proliferate faster than empty vector transfected cells (named MYST-mock). They also exhibit relatively high basal activation of the IP3 pathway. GHS-R1a full agonist (MK 677) strengthens basal IP3 pathway activation of MYST-Rg cells and mobilizes intracellular Ca^{2+}. Noteworthy, the basal IP3 pathway activation can be lessened by MSP treatment.
Thus, MSP could be a useful alternative to the current therapies of somatotroph adenomas as it inhibits constitutive IP-3 signalling, known as a significant hormonal release pathway, and promote apoptotic cell death.

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OC1.3 – ESE Young Investigator Award

Heterodimerisation of FSH and LH receptors positively modulates the LH-induced signalling profile

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The gonadotrophin receptors, LH receptor (LHR) and FSH receptor (FSHR) are G-protein coupled receptors, vital in regulating reproductive functions. During the follicular phase of the ovarian cycle, FSHR and LHR are separately localised to discrete cellular compartments, granulosa and theca cells respectively, where they control steroidogenesis and follicle maturation. However, as the follicle develops, LHR expression is induced in granulosa cells, resulting in temporary co-expression of FSHR and LHR in a single cellular compartment. Remarkably, little is known about the functional significance of this co-expression. While both FSHR and LHR are known to homodimerise, the question of whether FSHR and LHR can form functional heterodimers remains to be explored. Therefore, this study aims to determine if FSHR and LHR can form heterodimers and assess the functional impact on cellular signalling. The ability of FSHR and LHR to heterodimerise in live cells was observed via bioluminescence resonance energy transfer. Flow cytometric analysis indicated that co-expression of FSHR and LHR had no effect on cell surface targeting of either receptor. Furthermore, gonadotrophin-induced Gαlphas/cAMP signalling was not altered in the LHR/FSHR heterodimer compared to cells expressing each receptor alone. Interestingly, the pattern of LHR-induced Gαlphaq/calcium signalling was significantly altered in the presence of FSHR, from an acute and rapid signal to a more sustained calcium response. The prolonged calcium signal in LH-stimulated LHR/FSHR expressing cells appears to be mediated through activation of L-type calcium channels. A Gαlphai inhibitor, pertussis toxin, had no effect on calcium signalling, indicating there may be no alteration in G protein-coupling of the heterodimer. Co-expression of FSHR with LHR also enhanced the LH-induced MAPK signalling. The mechanisms underlying this change in signalling patterns will be further assessed. Overall this study indicates that LHR/FSHR heterodimers may represent a key mechanism for generating sustained signal responses in preovulatory follicles.

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OC1.4

BKM120, a pure PI3K inhibitor: a promising treatment for aggressive pituitary tumors or pituitary carcinomas

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Introduction
15% of pituitary tumors are considered as aggressive based on resistance to conventional treatment. Less than 40% of these cases respond to temozolomide treatment underlining the need for new therapeutic options. The PI3K/Akt/mTOR pathway, upregulated in different pituitary tumors subtypes, can be targeted by different drugs in particular BKM 120, a pure PI3K inhibitor, and BEZ235, a dual PI3K/mTOR inhibitor.

Objective
To study the anti-tumoral effect of BKM120 and BEZ235, on a model of rat prolactin pituitary tumor SMtTW-3.
Method
One-month after grafted SMtTW3 rats were treated via oral gavage 5 days/week with BKM120 (5 mg/kg per day, 20 days; n = 15) or BEZ235 (20 mg/kg per day, 15 days (n = 13) or 30 days (n = 13)) or control (n = 10 for each treatment). Antitumoral effect was evaluated by measuring tumor weigh at sacrificed and prolactin plasma level was measured before and after treatment. Ki67 index, mitosis were calculated on tumor section.
Results
BKM120 treatment reduces significantly tumor growth and prolactin secretion compare to controls with a final tumor weight of 5.36 ± 2.27 vs 28.76 ± 7.50 g ($P < 0.001$) and prolactin concentration of 1909 ± 234.6 vs 5465 ± 1026 μg/l.
BEZ235 treatment was less efficient and reduced tumor growth only after 30 days compare to control (35.87 ± 16.40 vs 47.52 ± 8.69 g; $P < 0.05$) and did not significantly decrease tumor growth after 15 days (10.89 ± 4.06 vs 11.86 ± 5.83 g) or prolactin secretion after 15 or 30 days of treatment (respectively 6718 ± 3687 vs 7183 ± 4452 μg/l and 6846 ± 3676 vs 8275 ± 4323 μg/l).
Both treatments were associated with a decrease of ki67 index and mitosis compared to tumors in control group.
Conclusion
BKM120, a pure PI3K inhibitor, present promising result for treating patient with aggressive pituitary tumor resistant to any conventional treatment. Differential effect between BKM120 and BEZ235 on tumor growth and PI3K/AKT/mTOR pathway is under investigation.

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OC1.5

Differential effect of octreotide treatment on expression of sstr2a, 3 and 5 in somatotroph adenomas

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Introduction
The effect of conventional somatostatin agonists (SA) on somatotroph pituitary adenomas is primarily mediated by somatostatin receptors (sstr) subtype 2. Sstr expression has been proposed as a prognostic marker for response to medical therapy. Earlier studies have shown lower sstr2a expression in SA treated adenomas, but selection bias could not be excluded.
Objective
To assess the effect of SA on the immunohistochemical expression of sstr2a, 3 and 5.
Patients and methods
Histological specimens from 78 somatotroph adenomas from patients operated for acromegaly at Oslo University Hospital, Rikshospitalet between 2000 and 2010 were evaluated. After exclusion of specimens without representative tumour tissue and from patients with pretreatment other than SA, 65 patients were included in the study. 28 of these were pretreated with SA (SA-group), 37 were operated directly (directsurgery-group; DS).
Immunohistochemical staining was performed with novel monoclonal anti-SSTR2A (UMB-1), anti-SSTR3 (UMB-5), and anti-SSTR5 (UMB-4) antibodies. The adenomas were scored into 12 categories by the IRS-score.
A subgroup of the 26 patients was earlier included in a randomised trial comparing direct surgery with surgery performed after SA treatment. Subgroup analyses were performed on this group.
Results
Immunohistochemical score for sstr2a in the pretreated patients (SA-group) was significantly lower than in patients operated directly (DS-group) ($P = 0.002$). Subgroup analysis in the randomised patients confirmed this finding. Scores for sstr3 and sstr 5 were not significantly different between groups. There was no significant difference in sstr 3 and 5 score between the treatment groups.
Conclusion
Sstr2 is down regulated by SA treatment. Subgroup analyses in the randomised cohort excluded selection bias concerning pretreatment. There is no major effect of SA treatment on sstr3 and sstr5.

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OC1.6

Management of euvolemic hyponatremia attributed to SIADH in the hospital: interim results from a prospective, observational, multi-center, global registry

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Introduction
Hyponatremia (HN) is the most common electrolyte disorder of hospitalized patients (pts). It occurs in up to 28% of in-pts, increases the in hospital risk of death by 1.47 fold, and is associated with significantly higher mortality risk following discharge. The HN Registry is the first large scale, international effort to document the clinical characteristics, treatments used, and impact of HN in hospital settings.
Methods
After informed consent or waiver, medical records of pts meeting the registry entry criteria were abstracted. Data are summarized by sample size (n) and percentage (%) for categorical data, and mean ± S.D. for continuous data.
Results
One thousand seven hundred and six euvolemic pts with SIADH enrolled at 157 US and 93 EU sites from Sept 2010 to Dec 2012 had sufficient data for analysis. The mean entry and discharge [Na^+] values for pts were 123.1 ± 5.5 and 131.8 ± 4.8 mmol/l. The table summarizes treatments given during hospitalization as treatment episodes of monotherapy (i.e. a treatment that was given alone during a discrete number of days).

Table 1

Treatment (Tx)*	During hospital stay (N)	Time to Tx from HN (days)	Duration of Tx (days)	(Na^+) Correction rate (mmol/l per day)	(Na^+) Increase > 12 mmol/l within 24 h of Tx (%)
No treatment	17% (285)	NA	NA	1.1 ± 1.3	2
Fluid restriction	56% (947)	1.70 ± 3.2	5.64 ± 5.7	1.4 ± 2.0	1
Normal saline	51% (862)	0.75 ± 2.1	2.67 ± 2.4	2.0 ± 2.8	2
Hypertonic saline	12% (212)	1.74 ± 3.1	2.37 ± 1.9	4.2 ± 4.0	11
Tolvaptan	17% (292)	3.44 ± 4.9	3.66 ± 5.0	5.2 ± 4.6	7

*pts could have > 1 treatment episode per hospitalization.

Conclusions
Fluid Restriction was minimally more effective than no treatment. Tolvaptan and hypertonic saline had the highest correction rates, but the latter was associated with a greater risk of overly rapid correction. More data is needed in this area to inform optimal clinical practice, which will be forthcoming from continuing analysis of the HN Registry.

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Bone & Calcium

OC2.1

Bone mass accrual following supplementation of vitamin D alone versus vitamin D + calcium in underprivileged Indian premenarcheal girls

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Objective
To determine effectiveness of supplementing vitamin D alone vs vitamin D + calcium on bone mass accrual in underprivileged Indian premenarcheal girls.
Methods
A double blind, matched pair, cluster randomization study was carried out in 200 premenarcheal girls (8–12 years) from three public schools. The participants were randomized into two clusters and were allocated to receive either vitamin D (Group A): 30 000 IU oral cholecalciferol every 3 months or vitamin D + calcium (Group B): 500 mg/day calcium and vitamin D 30 000 IU oral cholecalciferol every 3 months. The supplementation trial was done for the duration of 1 year. Anthropometry, biochemical parameters, total body bone area (TBBA), mineral content (TBBMC) and bone mineral density (TBBMD) by dual energy X-ray

absorptiometry were assessed at baseline and at the end of 1 year.
Results
At baseline vitamin D deficiency was observed in 84 (42%) girls. Post supplementation TBBMC, TBBMD and TBBA were significantly increased in both the groups in comparison to baseline. But the corresponding *Z* scores showed significant improvement only in group B. Mean percent increase in TBBMC was significantly higher in group B (from 841±174 to 1018±226 g, 22.3%) compared to group A (from 793±138 to 935±185 g, 17.6%, $P=0.02$). Improvement in TBBMC-for-age *Z* score was higher in the group B (from −1.1±0.9 to −0.9±0.9, 22%) vs group A (from −1.1±0.7 to −1.1±0.8, 13.6%, $P=0.03$). Similarly increments in TBBMD was significantly higher in group B (from 0.78±0.05 to 0.82±0.06 g/cm^2, 5.5%) vs group A (from 0.77±0.05 to 0.80±0.05 g/cm^2, 3.3%, $P=0.03$). However increase in TBBA was not significantly different between the two groups (14.4% in group B vs 13.8% in group A, $P>0.1$). No significant difference in mean percent increase in TBBMC were observed across vitamin D categories (<20, 20–30, >30 ng/ml) in both the groups. The increase in height was similar in the two supplemented groups (7.3±1.5 cm in group A vs 7.4±1.4 cm in group B).
Discussion
Low adult bone mass is linked to osteoporosis and fractures and is dependent on the extent of childhood and adolescent bone mineralization. Indices of bone health improved significantly following calcium and vitamin D supplementation.
Conclusion
Calcium along with vitamin D supplementation was more effective in improving bone mass accrual in underprivileged premenarcheal girls than vitamin D alone.
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OC2.2

Influence of vitamin D and calcium on reproductive hormones: a study in a VDR-ablated male mouse model and 300 healthy men
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Introduction
Vitamin D (VD) is metabolized locally in the testis, and ablation of the VD receptor ($Vdr^{-/-}$) in mice has proven a valid model for hereditary VD resistant rickets. However, only one of three published $Vdr^{-/-}$ strains presented with male infertility. Here, we investigated reproductive hormones, gene expression and the testicular histological phenotype of male $Vdr^{-/-}$ mice, and associated these results with reproductive hormone levels of men with different VD status.
Material and methods
In total 9 wildtype $Vdr^{+/+}$, 8 $Vdr^{-/+}$, and 11 $Vdr^{-/-}$ mice were investigated at the age of 10 and 15 weeks. Histology and gene-expression were examined in murine testis tissue by q-PCR and immunohistochemistry. Murine serum gonadotropins, testosterone and estradiol were measured with immunoassays. Serum levels of 25-hydroxyVD and calcium were subsequently established in a cross-sectional study of 300 men from the general population and associated with serum concentrations of estradiol, testosterone, Inhibin B, SHBG, LH and FSH.
Results
Testicular histology was grossly normal in $Vdr^{-/-}$ mice without differences in tubular diameter, epithelial width or Leydig cell-numbers. No changes were found in serum hormone levels and accordingly, no difference in expression of *Cyp19a1, Erα, 17-βhsd, Star, Inhibin B, and Amh*. However, a significantly lower expression of *Erβ* in testis and epididymis ($P<0.05$) was found in $Vdr^{-/-}$ and $Vdr^{-/+}$. In the human study, 44% were VD insufficient (<50 nM), and 25-hydroxyVD correlated positively with SHBG and negatively with free androgen index (FAI) ($P<0.05$), while albumin-corrected calcium had the opposite effect. Adjusted regression analyses confirmed the associations of VD and calcium with SHBG and FAI ($P<0.05$).
Conclusion
VD is apparently dispensable for testicular hormone biosynthesis in mice and humans, but may be regulating Erβ expression in murine testis and epididymis. The associations between serum 25-hydroxyVD and calcium with SHBG /FAI in men indicate a systemic rather than testicular effect of VD.
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OC2.3

Screening for GNAS genetic and epigenetic alterations in progressive osseous heteroplasia: first Italian series
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Progressive osseous heteroplasia (POH) is a rare autosomal dominant disorder of mesenchymal differentiation characterized by progressive heterotopic ossification (HO) of dermis, skeletal muscle and deep connective tissues. Initially HO occurs during infancy as osteoma cutis, then extends progressively into deep connective tissues during childhood. Most cases of POH are caused by paternally inherited mutations of *GNAS* gene. Maternal mutations as well as epigentic defects of the same gene lead to pseudohypoparathyroidism (PHP) and Albright's hereditary osteodystrophy (AHO). Recently, some reports documented the existence of POH patients showing additional features characteristics of PHP/AHO. Thus, POH has been proposed to be part of the spectrum of HO disorders caused by inactivating *GNAS* mutations.
We investigated nine unrelated POH patients, one of whom also showed resistance to PTH and TSH, for GNAS genetic and epigenetic status by direct sequencing and MS-MLPA. In a subset of patients, we performed RNA segregation analysis in order to establish the parental origin of the mutated allele. We detected Four *GNAS* mutations in five of nine patients, all *de novo* and predicting truncated proteins. In three mutated patients, we demonstrated that the mutation occurred on the paternal allele. No evident differences were observed among patients harboring different mutations, as well as between mutated and non-mutated patients. Thus, neither the presence/absence nor the type or the localization of the mutation allowed to predict a specific phenotype or the severity of progression within the spectrum of *GNAS*–related disorders. All tested POH patients resulted wild type for *GNAS* imprinting status and no copy number abnormalities were found.
In conclusion, our results support that POH belongs to a continuum of HO disorders associated with inactivating *GNAS* mutations and further expand the spectrum of associated genetic defects. Moreover, unlike PHP, methylation alterations at *GNAS* locus are absent or uncommon in POH.
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OC2.4

Natural course of changes in bone mineral density after orthotopic liver transplantation: up to 5 years follow-up in a single centre
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Introduction
Low energy trauma fractures are prevalent in end-stage liver disease and after orthotopic liver transplantation (OLT). However, data on bone mineral density (BMD) are scarce in these patients. In this study, we evaluated the natural course of changes in BMD after successful OLT.
Study design
All recipients of a successful OLT between 2000 and 2011 from the Leiden University Medical Centre, in whom BMD data were available, were included. Patients treated with bisphosphonates were censored at start of bisphosphonate therapy (last observation carried forward). Follow-up duration was five years.
Results
The cohort consisted of 223 patients, 69% men, mean age of 50 (17–70 years). Most common primary liver pathology was viral (31%) or alcoholic liver disease (23%). All patients received prednisone, the majority either tacrolimus or cyclosporine. At screening, osteoporosis or osteopenia were found in 18 and 38% of patients at the lumbar spine (LS) and in 10 and 45% of patients at the femoral neck (FN). BMD declined significantly at both sites 6 months after OLT, but increased thereafter at the LS, reaching pre-transplant values at 2 years, and subsequently stabilizing. In contrast, FN BMD declined from 6 months onward, remaining consistently lower than pre-transplant values. The cumulative incidence of osteoporosis at five years after OLT was 14.6% at the LS and 19.6% at the FN.
Conclusion
Osteoporosis and osteopenia are prevalent in patients with end-stage liver disease. An overall decline in BMD is observed within the first 6 months after OLT, with subsequent recovery to pre-transplant values at the LS, but with further decline at the FN. Further investigations are currently underway to elucidate the potential

factors responsible for bone loss before and after OLT and to examine the predictive value of changes in BMD for risk of fracture in these patients.

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OC2.5

Genetic analysis of *CDKN1B* gene in familial primary hyperparathyroidism

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Primary hyperparathyroidism (PHPT) is usually a sporadic disorder, but in $<10\%$ of cases occurs as part of hereditary syndromes, including multiple endocrine neoplasia types 1 and 2A (MEN1 and MEN2A), hyperparathyroidism-jaw tumor syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP).
MEN 1 is an autosomal dominant disorder characterized by tumours in multiple endocrine glands, most commonly parathyroid, enteropancreatic and anterior pituitary glands. To date, germline mutations in the *MEN1* gene have been identified in 70–80% of *MEN1* kindreds. FIHP has a heterogeneous molecular ethiology, since germline mutations in *MEN1*, *HRPT2* and *CASR* genes have been reported. Recently, germline mutations of cyclin dependent kinase inhibitor 1B (*CDKN1B*) gene, encoding the p27 protein, have been identified in 8 kindreds with MEN1 syndrome which were negative to the MEN1 genetic screening.
The aim of this study was to perform a genetic screening of *CDKN1B* gene in patients with MEN1 syndrome and FIHP (33 and 17, respectively). All MEN1 and FIHP probands were negative for MEN1 gene mutations at genetic testing.
Genomic DNA from index cases was analyzed by PCR amplification of the entire coding region and splice sites, and direct sequencing was performed by a 16-capillaries automatic sequencer.
A novel frameshift germline mutation in *CDKN1B* gene, c.372_373delCT/p.Asn124AsnfsX2, was identified in a MEN1 proband. A construct expressing p27_c.372_373delCT was generated to assess the functional properties of the mutant protein *in vitro*. Indirect immunofluorescence demonstrated that the mutant protein is mainly retained in the cytoplasm, affecting the cell cycle inhibitory function of p27 in the nucleus. Our results confirm that germline CDKN1B mutations are involved, althought rarely, in parathyroid tumorigenesis.

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OC2.6

Hypomineralized teeth as biomarkers of exposure to endocrine disruptors

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MIH for Molar Incisor Hypomineralization is a recently described pathology affecting around 18% of six year old children. Although a number of putative factors have been hypothesized, etiology of MIH remains unknown. The parallel increase of exposure to endocrine disruptors (EDs) and the prevalence of MIH led us to investigate a possible relationship between both events.
Rats were orally exposed daily to low dose of bisphenol A (BPA), genistein, vinclozolin, alone (for BPA) or in combination, from the conception to the sacrifice, mimicking human environmental exposure. Macroscopic observation of male rat incisors showed that the phenotype induced by BPA was the most evident with 75% of rats presenting random opaque white spots comparable to those observed in human MIH, whereas only 50% of GEN and VINCLO treated rats shared similar phenotype. Human MIH and BPA treated rat teeth were analyzed in parallel by scanning electron microscopy (SEM) - Energy dispersive X-ray (EDX) and histology. Both of them exhibited the same hypomineralization phenotype. BPA targeted specifically the expression of two major enamel genes, enamelin and kallikrein 4 (Klk4) at the transcriptional level. Rat ameloblastic HAT-7 cells were stably transfected with plasmids containing KLK4 promoter, and treated with 1 nM BPA, 1 nM GEN, 1 nM VINCLO. BPA decreased both KLK4 mRNA level and KLK4 promoter activity. Conversely, GEN increased KLK4 expression whereas VINCLO had no effect on this gene, a possible reason for the lesser effect on enamel hypomineralization.
Our data strongly support a role for EDs acting as BPA in MIH pathology. In conclusion, MIH teeth may represent a much needed early biomarker, easily accessible, for ED exposure in humans.

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Thyroid

OC3.1

Targeting of *PATZ1* by miR-29b is a downstream effect of oncogenic Ras signalling in thyroid cells

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PATZ1, a member of the POZ-ZF protein family of transcription factors is emerging as an important cancer-associated factor that can act either as oncogene or tumour-suppressor depending on the cellular context. Consistent with a tumour-suppressor role in thyroid cells, we have shown that PATZ1 is highly downregulated in anaplastic thyroid carcinomas compared to normal thyroid tissue and is a powerful inhibitor of anaplastic thyroid cancer cell survival, migration, invasiveness and tumorigenicity.
Looking for the upstream signalling pathway regulating PATZ1 expression in thyroid cells, we searched for microRNAs targeting PATZ1. In order to identify miRNAs predicted to bind the 3′UTR of PATZ1 we used bioinformatics free tools, based on the miRanda application and the mirSVR predicted target site scoring method. Among the miRNAs identified by this analysis we validated miR-29b. Indeed, we demonstrated that it is able to target PATZ1 and cause downregulation of PATZ1 expression at both mRNA and protein level in different cell systems, including rat thyroid cells. Interestingly, miR-29b is induced by Ras during transformation of FRTL-5 rat thyroid cells toward an undifferentiated phenotype, resembling that of anaplastic carcinomas and characterized by the acquisition of a migratory and invasive behaviour. In these cells, we observed a strong down-regulation of PATZ1 expression, which starts as early as 2 h after Ras induction, and an inverse correlation between the expression of miR-29b and PATZ1 mRNA and protein levels.
These results are consistent with the suppressor role of PATZ in thyroid carcinogenesis and suggest that down-regulation of PATZ1 expression, through miR-29b, may be a downstream effect of the oncogenic Ras signalling in thyroid cell transformation.

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OC3.2

Mitochondrial mass and function is regulated by PI3K signaling in thyroid cancer cells

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Objective
Abnormal mitochondria are well known in oxyphilic thyroid tumors but recent data also confirm profound mitochondrial alterations in other thyroid carcinomas. These changes are linked to the aggressiveness of the tumors. Our group recently demonstrated in an *in vivo* model, that inhibition of phosphoinositide 3-kinase (PI3K) signalling suppressed the invasive and metastatic behaviour of thyroid cancer cells. Here, we evaluated whether a modulation of PI3K signalling changes mitochondrial mass and function.
Methods
We used follicular (FTC-133, WRO) and anaplastic (8505C) carcinoma cell lines to characterize mitochondrial mass and function both under baseline conditions and inhibition of PI3K signaling. Therefore, we transfected phosphatase and tensin homolog (PTEN) mutated FTC-133 cells with *wild type* PTEN or empty vector. We compared these chronic effects with an acute inhibition of PI3K signaling over 18 h using the pan-PI3K inhibitor GDC-0941 in all cell lines.

Results
Mitochondrial mass remained unchanged in all cell lines but active mitochondria were significantly decreased by acute PI3K inhibition. This effect was most pronounced in FTC-133 cells. It was accompanied by a significant shift in oxygen consumption (directly measured by Clarke electrode), a decrease in the production of reactive oxygen species (Dihydrorhodamine 123 and MitoSOX) and reduced protein carbonylation (OxyBlot). Evaluation of proteins involved in mitochondrial biogenesis (PGC1α) and mitochondrial autophagy (BNIP3 and Beclin1) indicate a shift in the balance of biogenesis and autophagy upon acute PI3K inhibition. Electron microscopy confirmed a major change in mitochondrial ultrastructure and an increased number of lysosomes/autophagosomes.
Summary
Our data demonstrate that changes in PI3K signaling alter mitochondrial function. These functional data suggest a major impact of the PI3K cascade on the reprogramming of mitochondria in cancer. These mitochondrial alterations may have important consequences to explain the aggressiveness of thyroid cancer subtypes with activated PI3K.

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OC3.3 – ESE Young Investigator Award

SP600125, a new p53 selective anticancer drug: effects on poorly differentiated thyroid cancers

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Poorly differentiated thyroid cancers are associated with variable types of p53 function derangements and bad prognosis due to the lack of effective treatments. SP600125 is a widely used JNK-inhibitor which recently showed anticancer properties in a p53 related way. Here, we tested the effect of SP600125 on four different thyroid cancer cell lines derived from PDTCs and ATCs with different p53 status.
We analyzed the effects on cellular replication and apoptosis, changes in morphology or intracellular pathway activities.
Our results show that SP600125 is able to suppress the growth of the three p53-mutated cell lines but not of the p53-null one with significant results after 48 h of incubation and EC50 of 1.3–8.4 μM. This effect is accompanied by a slight caspase 3 activation confirmed by functional assay and western-blotting.
The growth inhibition is correlated with an increase of p21 expression levels of more than 3-fold and a 2-fold increase in nuclear dimensions, thus consistent with significant effects on cell cycle progression and DNA accumulation.
Moreover, we observe that SP600125 treatment in the p53-mutated cells causes polymerized tubulin stabilization, as shown by the relative increase of polymer versus monomer form; this is accompanied by increased polymerized tubulin acetylation, a marker of microtubules stability. Confocal microscopy showed morphological alteration of treated cells as well as differences in acetylated tubulin distribution, with a switch from physiological perinuclear organization to cytoplasmic distibution. These alterations correlate with an increase of cytoplasmic and nuclear area, with variations in p53 and JNK subcellular localization.
All together these data confirm that SP600125 acts in a p53-dependent way and it's able to induce cell growth arrest with significant effects on nuclear and cell division, microtubule organization and intracellular protein distribution in a subgroup PDTC cells. SP600125 appears a reliable candidate drug for PDTCs harbouring p53 mutations.

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OC3.4

DNA methylation signatures identify biologically distinct thyroid cancer subtypes

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Introduction
The global patterns of aberrant DNA methylation in thyroid cancer are not known. In this study, we have used DNA methylation arrays to determine, for the first time, the genome-wide promoter methylation status of different subtypes of thyroid tumors.
Material and methods
We analyzed a cohort of 54 snap frozen thyroid samples (thirteen papillary tumors, six follicular, eleven anaplastic, twenty medullar and four normal tissue samples). Also human thyroid carcinoma-derived cell lines, K1, FTC-133, 8305C and TT were obtained from the ECACC to analyze. A microarray-based DNA methylation profiling was performed with the Illumina Infinium Human Methylation 27K Platform with some of these samples. Hierarchical cluster analysis and definition of differentially methylated genes, computational gene expression analysis and gene ontology analysis of differentially methylated genes were preformed. To confirm those data, bisulfite pyrosequening of several genes were also done. Also proportions of PcG-marks and bivalent domains in different methylation groups were studied.
Results
We identified 262 and 352 hyper and 13 and 21 hypomethylated genes in differentiated papillary and follicular tumors respectively. Surprisingly, undifferentiated tumors displayed more hypomethylated genes (280 in anaplastic, 393 in medullar) than aberrantly hypermethylated genes (86 in anaplastic, 131 in medullar). Hypermethylated genes in tumors originating from follicular cells were preferentially involved in developmental processes whilst those in medullary tumors were primarily involved in cell signaling and iodium pathways. Hypomethylated genes were enriched in immune system functions. Among the genes identified, we show that four potential tumor suppressor genes (*ADAMTS8*, *HOXB4*, *ZIC1*, and *KISS1R*) and four potential oncogenes (*INSL4*, *DPPA2*, *TCL1B*, and *NOTCH4*) are frequently regulated by aberrant methylation in thyroid tumors. Further studies are needed to determine the potential clinical interest of the tumor subtype-specific DNA methylation signatures described herein and the role of aberrant promoter hypomethylation in undifferentiated thyroid tumors.

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OC3.5

Role of the calcium-calmodulin dependent kinase 2 in medullary thyroid carcinoma

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Recent studies demonstrated that the calcium/calmodulin dependent kinase 2 (CaMKII) is involved in the regulation of proliferation and survival of epithelial cells, were it phosphorylates RAF-1 and modulates MAPK pathway. A endogenous CaMKII inhibitor (hCaKIINα) is expressed in some cell types. It is down-expressed in colon and in ovarian cancer where it inversely correlates with the disease extension.
Aim of our study is to determine the possible role of CaMKII in the medullary thyroid carcinoma (MTC), to determine whether hCaKIINα is expressed, and whether its level of expression correlates with the clinicopathological features of MTC.
To this purpose, RET^{C634Y} e RET^{M918T}, two RET mutants most frequently found in MTC, were expressed in NIH-3T3 cells and the activation status of CaMKII was determined. Oncogenic RET expression in serum-starved NIH-3T3 mutants induced maximal CaMKII activation. In two MTC cell lines (TT and MZCRC-1 carrying the same RET mutants) CaMKII was activated. Inhibition of CaMKII in these cells induced a reduction of ERK phosphorylation and cell proliferation. Then, the expression of hCaKIINα RNA was determined by real-time PCR in 24 primary MTCs and was correlated with some clinicopathological parameters. Gender and age at diagnosis did not correlate with hCaKIINα RNA expression. Serum calcitonin, ($R^2 = 0.032$, $P = 0.017$ by Spearman rank correlation), tumor volume ($P = 0.0094$ by ANOVA), lymph node metastasis ($P = 0.0297$ by *t*-test) and staging ($P = 0.0043$ by ANOVA) were negatively correlated with the hCaMKIINα mRNA expression.
In conclusion, CaMKII is activated by RET mutants and is activated at baseline in MTC cell lines were it mediates the oncogenic pathway leading to cell proliferation. The mRNA expression of its endogenous inhibitor hCaKIINα inversely correlates with the severity of MTC. CaMKII might represent a new target for MTC therapy.

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OC3.6 – ESE Young Investigator Award

Subclinical hyperthyroidism and risk of cardiovascular and all-cause mortality

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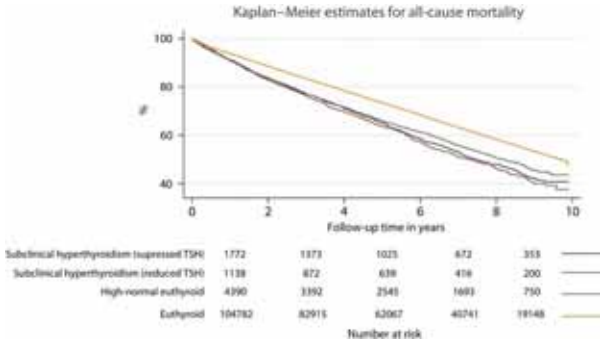
Background
It is still discussed if subclinical hyperthyroidism and 'high-normal' thyroid function are risk-factors for cardiovascular mortality.
Objectives
To examine the risk of cardiovascular and all-cause mortality in relation to subclinical hyperthyroidism.
Methods
Patients consulting their general practitioner from 2000 to 2009 in Copenhagen, Denmark, who underwent thyroid blood tests, were identified by individual-level linkage of nationwide registries. Patients with a history of thyroid disease or related medication were excluded. Risk of cardiovascular mortality was analyzed using Kaplan–Meier curves and Poisson regression models were applied to estimate Incidence Rate Ratios (IRR).
Results
Of 574 595 included individuals (mean age 48.7 years (s.d. ±18.3); 39.1% males) 550 927 (95.9%) were euthyroid, 1603 (0.3%) had overt hypothyroidism, 11 834 (2.1%) subclinical hypothyroidism, 3967 (0.7%) overt hyperthyroidism and 6264 (1.1%) subclinical hyperthyroidism. Increased risk of cardiovascular mortality was found in two levels of subclinical hyperthyroidism (TSH <0.1, 0.1–0.2 mU/l and normal free thyroixine): IRR 1.24 (95% CI: 1.09–1.41), IRR 1.21 (1.09–1.34) and in 'high-normal' levels of euthyroidism (TSH 0.2–0.4 mU/l): IRR 1.21 (1.13–1.29).



Conclusions
Subclinical hyperthyroidism and 'high-normal' thyroid function are significant risk-factors for cardiovascular and all-cause mortality.

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Adrenal

OC4.1

Pre-clinical model detects castrate-resistant cancer repopulating cells in localised prostate tumours

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Introduction
Lack of clinically relevant experimental models of human prostate cancer hampers evaluation of novel therapeutic agents. Currently, androgen deprivation therapy is the gold standard treatment for advanced prostate cancer, but inevitably cells survive and repopulate the tumour. Castrate-resistant cells are critical therapeutic targets for more effective treatments but current model systems cannot determine when they arise in disease progression and are unable to recapitulate variable patient response to treatment.
The aim of this study was to develop stromally supported xenografts from multiple patients with early stage-localised disease to investigate the castration response. It was postulated that prior to the development of aggressive/metastatic disease, localised prostate cancer specimens may already harbour castrate-resistant, cancer repopulating cells.
Methods
To test this concept a reproducible and reliable model was used to establish 12 patient localised prostate cancers *in vivo*. Using these 12 engrafted tumours, response to short and long term castration, as well as androgen restoration was examined. Tumour grafts were analysed using pathology, proliferation/apoptotic indices and biomarker expression.
Results
Histopathology of all 12 engrafted tumours mimicked that of the original tumours and when host mice were castrated, the tumours regressed showing significant changes in proliferation and apoptosis. Four weeks thereafter, a population of growth quiescent luminal cells, expressing low AR and PSA remained, which expressed variable immuno-positivity for common stem cell markers Nkx3-1, CD44, ALDH1 and NANOG. These castrate-resistant cells showed regenerative capacity when testosterone was re-administered to castrated hosts, and restoration of normal AR and PSA expression.
Conclusions
Stem-like castrate-resistant (but hormone sensitive) tumour cells were detected in localised prostate cancer specimens from which tumours regenerate; thus proving the need to further characterise these cells and elucidate common pathways to therapeutically target them.

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OC4.2

Single nucleotide polymorphism array profiling of adrenocortical tumors: evidence for an adenoma carcinoma sequence?

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Background
Adrenocortical tumors consist of benign adenomas (ACA) and highly malignant carcinomas (ACC) with a still incompletely understood pathogenesis. Our aim was to test, whether there is evidence for an adenoma-carcinoma sequence.
Patients and methods
High-resolution single nucleotide polymorphism (SNP) microarrays (Affymetrix SNP 6.0) were used to detect copy number alterations (CNAs) and copy neutral losses of heterozygosity (cnLOH) in 46 adrenocortical tumors (24 ACA and 22 ACC) matched with normal samples.
Results
Genomic clustering showed good separation between ACA and ACC samples, with the best partition for chromosome (chr) 5, which was highly amplified in 17/22 ACC. Of note, the median number of CNA per tumor was not significantly different between ACA and ACA (43.5 vs 132; P=n.s.). However, recurrent CNAs (observed in >2 samples, frequency >15%), the median number of large CNAs (>100 Kb) or CN losses, and the percentage of samples affected by cnLOH were significantly higher in ACC (3993 vs 98 recurrent CNAs; 62.5 vs 7 large CNA; 72.5 vs 5.5 CN losses; 90.9 vs 29.1% cases with cnLOH, $P<0.05$ for each). Interestingly, more than 70% of alternations found in ACA were also present in ACC, most of them being observed in chr 5p15.33, 9q32–34, 16p13.3, and 19p13.3 and involving among others 11 growth factors, 41 transcription factors, 16 protein kinases, and 11 oncogenes. In addition, Notch signaling was the most frequently altered pathway in both tumor entities. Finally, a CN gain at chr 11p15.5 ('IGF2 locus') appears to be an early alteration in a multi-step tumor progression (present in 25% of ACA), followed by loss of one allele leading as a second hit to a cnLOH with consecutively increased expression of the imprinted IGF2 gene only in malignant adrenal tumors.
Conclusion
Our results on genetic alterations support the concept of an adenoma-carcinoma sequence in adrenocortical carcinoma.

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OC4.3

Prognostic factors of advanced unresectable by stage III and IV ENS@T adrenocortical carcinomas (ACC)

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Introduction
The prognosis of stages III–IV ACC patients is dismal. The 5-year survival of stage IV ACC ranges between 0 and 13% and no prognostic study has focused on stage III, yet. Several reports suggest a greater heterogeneity of advanced ACC prognosis that initially thought.
Aim
The primary objective of our study was to analyse the prognostic factors of overall survival of the subgroup of advanced unresectable stage III–IV ACC patients collected in the ACC-ENS@T registry.
Methodology
The primary end-point was to determine the median overall survival (OS). Secondary objectives were: 1, 2, 5 year-OS, to refine the prognostic stratification, to analyse the role of early ACC management and delays in treatment. All relevant clinical parameters as well as therapeutic management were captured. Weiss score and ki67 index performed in a subgroup of patients (236 and 122, respectively) were analyzed in a second model.
Patients
Three hundred and thirty-four adult patients were enrolled (100 stage III and 234 stage IV ACC) treated between 2000 and 2009. Inclusion criteria were: age >18 years, unresctable ACC (R1, R2, Rx), clinical and follow-up data available.
Results
After a median follow-up of 60 months two hundred and fifty patients (74%) died. Median OS was 20 months. The 1-, 2- and 5-years survival rates were 67, 42 and 19%.
At multivariate analysis, age >50 years (HR $=1.4$, $P=0.02$), glucocorticoids secretion (HR $=1.3$, $P=0.04$), number of tumor organs >3 (HR $=3.2$, $P<0.0001$), adipose infiltration (HR $=1.8$, $P=0.0009$) and adrenalectomy (HR $=0.4$, $P<0.0001$) were significantly associated with the risk of death. When pathogical parameters were entered in the MV model, only Ki-67 $>20\%$ HR $=3.6$, $P=0.001$) remained significant.
Conclusion
This large ENSAT prognostic study allows to propose a prognostic stratification of advanced ACC patients that will drive future therapeutic approaches. Surgery of adrenal primary is confirmed as a major therapeutic intervention. Ki 67 determination becomes mandatory.

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OC4.4

Neuronal dysfunction in the hippocampi of cured Cushing's syndrome patients, detected by 1H-MR-spectroscopy

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Introduction
Proton magnetic resonance spectroscopy (^{1}H-MRS) is a sensitive, non-invasive imaging technique capable of measuring brain metabolites *in vivo*. Chronic exposure to endogenous hypercortisolism in Cushing's syndrome (CS) is associated with negative effects on memory and hippocampal volumes, even after biochemical cure.
Objective
To investigate metabolites in the hippocampi of CS patients and controls, using ^{1}H-MRS.
Patients and methods
Eighteen right-handed cured CS patients (age 44.8 ± 12.5 years, 12.6 ± 3.8 years of education), 18 right-handed healthy controls, matched for age (40.0 ± 11.9) and years of education (14.4 ± 3.8) underwent 3-Tesla magnetic resonance imaging (3T MRI) and ^{1}H-MRS including the head of each hippocampus. Concentrations of Glu (Glutamate), Glx (Glutamate + Glutamine), NAA (NAcetyl-aspartate), total-NAA (NAcetyl-aspartate + N-Acetyl-aspartyl-Glutamate), Cho (Glycero-phosphocholine and Phosphocholine compounds), Cr (Creatine) and MI (mionositol) were measured (mmol/l). Hippocampal volumes were additionally calculated using an automated procedure (Freesurfer).
Results
CS patients had lower NAA than controls in the left and right hippocampus (5.2 ± 1.0 vs 6.1 ± 0.7, $P<0.05$; 4.9 ± 0.8 vs 6.1 ± 0.6, $P<0.001$ respectively), and lower total-NAA in the right (5.7 ± 0.9 vs 6.3 ± 0.9, $P<0.05$), suggesting neuronal dysfunction/loss. CS patients had higher Glx than controls in both hippocampi (10.4 ± 1.9 vs 8.6 ± 1.4, $P<0.01$; 9.9 ± 1.6 vs 8.9 ± 1.3, $P<0.05$ respectively), suggesting glial proliferation, as a repair mechanism after neuronal dysfunction. No differences were found in the other brain metabolites, and there were no differences in left (3815.78 ± 502.96 mm^3) and right (3980.75 ± 369.44 mm^3) total hippocampal volumes between CS patients and controls (3945.08 ± 408.90 and 4108.39 ± 365.11 mm^3, respectively).
Conclusion
Persistently abnormal metabolites are evidenced in the hippocampi of CS patients despite endocrine cure. These functional alterations could be early markers of glucocorticoids neurotoxicity and would precede hippocampal volume reduction.

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OC4.5 – ESE Young Investigator Award

Influence of somatic mutations on the lateralization index of adrenal vein sampling in aldosterone producing adenomas

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Adrenal vein sampling (AVS) is a technically demanding procedure required for identification of suitable candidates for unilateral adrenalectomy in primary aldosteronism. Recently, somatic mutations in aldosterone producing adenomas (APA) have become a focus of research. We identified KCNJ5 K^+-channel mutations in 34% of APAs, and more recently mutations in 2 ATPase family members in 11 and 6%. However, it is unclear whether steroid gradients during the AVS are influenced by the mutation status of the APA. This might have impact on final diagnosis and treatment.
To analyse the influence of somatic mutations on gradients in AVS, 39 patients with APAs diagnosed according to the German Conn Registry standard were studied. All subjects underwent technically successful AVS (with a selectivity index >2) and had a mutation analysis in tumor tissue. The mutation status of the APAs was: 11 KCNJ5 mutations (6 G151R, 5 L168R), 8 ATPase mutations (5 ATP1A1, 3 ATP2B3) and 20 with none of these mutations.
Patients with ATPase mutations showed higher blood pressure and lower potassium levels than those with KCNJ5 mutation or no mutation. Lateralization indexes of ATPase mutation carriers were 55.8 ± 23.4 compared to 27.8 ± 11.6 in KCNJ5 mutation carriers and 27.7 ± 9.1 in KCNJ5/ATPase negative patients ($P=$ns). Contralateral suppression (ratio of plasma aldosterone to cortisol of the contralateral side and aldosterone to cortisol in the periphery) was most distinct in ATPase mutated patients with marginal significance (ATPase 0.2 ± 0.1, KCNJ5 0.6 ± 0.2, KCNJ5/ATPase non-mutated 0.8 ± 0.2, $P=0.065$).
In summary, ATPase mutations seem to be associated with a higher lateralization of aldosterone production. In contrast to prior findings we did not confirm higher lateralization indexes in KCNJ5 mutated patients. However, further studies with a higher number of patients being included are required.

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OC4.6

9-*cis* retinoic acid, a novel treatment option for adrenocortical cancer? *in vitro* and *in vivo* studies

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Background
The medical treatment options of adrenocortical cancer are limited. In our previous meta-analysis of adrenocortical tumor genomics data, adrenocortical cancer was found to be associated with reduced retinoic acid production and retinoid X receptor-mediated signaling.
Objective
To study the potential antitumoral effects of 9-*cis*-retinoic acid (9-*cis*RA) on the adrenocortical cancer cell line NCI-H295R and in a xenograft model.
Methods
Cell proliferation (flow cytometry), hormone secretion (cortisol, dehydroepiandrosterone, aldosterone) and gene expression (microarray) have been studied in the NCI-H295R cell line. A complex bioinformatics approach involving pathway and network analysis and comparison to previously published microarray studies have been performed. Selected genes were validated by real-time qRT-PCR. Athymic nude mice xenografted with NCI-H295R were used in a small pilot *in vivo* xenograft model for the study of 9-*cis*RA on tumor growth.
Results
9-*cis*RA significantly decreased cell viability and steroid hormone secretion in a concentration- and time-dependent manner in the NCI-H295R cell line. Four major molecular pathways have been identified by the analysis of gene expression data. Ten genes involved in: i) steroid hormone secretion (*HSD3B1*, *HSD3B2*), ii) retinoic acid signaling (*ABCA1*, *ABCG1*, *HMGCR*), iii) cell cycle damage (*GADD45A*, *CCNE2*, *UHRF1*) and the iv) immune response (*MAP2K6*, *IL1R2*) were successfully validated. 9-*cis*RA appears to directly regulate cell cycle by network analysis. In the *in vivo* xenograft model 9-*cis*RA reduced tumor growth, as well.
Conclusions
9-*cis*RA might represent a promising new candidate in the treatment of hormone-secreting adrenal tumors and adrenocortical cancer.

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Reproduction

OC5.1

BMP15-dependent gene-expression profiling in human granulosa cells

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The oocyte-derived growth and differentiation paracrine factor BMP15 has emerged as an essential regulator of the ovarian folliculogenesis, from evidences in animal models (knockout mice and sheeps with naturally occurring mutations) and humans. Indeed, several BMP15 mutations have been identified in association with primary ovarian insufficiency (POI), a heterogeneous and frequent fertility disorder characterized by the premature depletion of ovarian follicles in women <40 years. Despite the frequent inheritability of POI, genetic alterations still explain only few cases and we hypothesized that the elucidation of the BMP15-dependent effects on granulosa cells (GCs) would give interesting insights for advancements in this direction. Through a global approach for large-scale gene-expression profiling, the analysis of the transcriptome induced by recombinant human BMP15 action on primary cultures of human Granulosa Cells (GCs) was performed. The GCs were obtained from fertile women undergoing IVF and then stimulated in triplicates with BMP15. Treated and untreated GCs were harvested after 0, 2 and 6 h, to evaluate early and late regulated genes. RNAs from each condition were processed for hybridization on Illumina beadchips. Statistical analysis identified about 100 differentially expressed genes (FDR q-value <0.001) already 2 h after treatment (cutoff fold-change >2). As expected, we found that BMP15 induces genes important for the modulation of the BMP/TGFbeta signaling. Interestingly, BMP15 inhibits the GCs expression of genes important for ovarian physiology (follistatin, TGFB3R, steroidogenesis enzymes and metalloproteases family members) and regulates apoptotic and proliferative genes (BCL and MAPK families), thus indicating BMP15 as a master regulator of folliculogenesis processes. In conclusion, this is the first comprehensive panel of transcriptomic effects induced by BMP15 on human GCs. The analysis of such regulated genes should allow the selection of novel candidates to be screened in POI cohorts for the comprehension of the pathogenic mechanisms underlying POI.

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OC5.2

The (*TAAAA*)n polymorphism in the SHBG gene is related to prenatal androgenization of female fetus: possible implications of the developmental origin of metabolic disorders

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Introduction
The aim of this study was to examine whether the distribution of SHBG (*TAAAA*)n repeat variants contributes to the exposure of the female fetus to androgen excess, by influencing the, *in utero,* androgen availability.
Methods
The study population consisted of 100 pregnant women that carried female fetuses and underwent the procedure of amniocentesis due to age (older than 35). Blood samples and amniotic fluid samples were drawn for the measurement of SHBG, testosterone (T) and estradiol (E_2). DNA was extracted from peripheral blood leucocytes and amniotic cells and the SHBG (*TAAAA*)n was genotyped by PCR. 53 of the women enrolled in the study population went into labor few weeks later in the same Department. The exact birth weight of their female neonates was recorded.
Results
Women with long SHBG alleles (8/8, 8/9, 8/10, 9/9, 9/10, 10/10) presented lower levels of SHBG in the amniotic fluid compared to those with short alleles (6/6, 6/7, 6/8, 7/7, 7/8) ($P=0.027$). Moreover, homozygous for the allele 8, 9 and 10 *TAAAA* repeats (8/8, 9/9, 10/10) had higher T/SHBG ratio in amniotic fluid than heterozygotes and those with short alleles ($P=0.016$). A positive correlation between the birth weight of the female neonates and maternal SHBG was also recorded ($r=0.292$, $P=0.032$).
Conclusion
The presence of long $(TAAAA)_n$ SHBG alleles is associated with lower SHBG levels and higher T/SHBG ratio in the amniotic fluid of female fetuses at midgestation. In addition, lower levels of maternal SHBG are related to lower birth weight of female neonates. Thus, SHBG variants may provide a genetic link to the developmental origin of metabolic disorders and in particular to that of PCOS in women.

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OC5.3

Age-related hormonal and metabolic alterations in women with polycystic ovary syndrome (PCOS)

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Introduction
PCOS-related hormonal and metabolic alterations seem to persist later in life. The aim was to investigate age-related hormonal and metabolic parameters in PCOS and in healthy women in order to determine cut-off values predicting PCOS.

Subjects and methods
PCOS women and 230 healthy controls were divided accordingly: 18–24, 25–29, 30–34, 35–39, 40–44, 45–49 and >50 years. A particular interest was to analyze serum testosterone (T) levels using the golden standard method, liquid chromatography-tandem mass spectrometry (LC-MS).
Results
In PCOS group, there was a U-shaped pattern (decrease towards menopause and increase thereafter) in serum androgens and free androgen index (FAI) while in controls a significant decrease with age in dehydroepiandrostendione sulphate and androstenedione (A), and an increase in FAI were observed. T and FAI were significantly higher in PCOS compared to controls in age groups 18–38 years and 18–44 years, respectively ($P<0.001$, BMI adjusted).
Serum insulin levels and HOMA-IR were significantly higher in PCOS in all age groups except 45–49 years however, the differences disappeared after BMI adjustment except in women >50 years (12.9 vs 5.7 mU/l, $P=0.02$). Triglycerides were higher and HDL tended to be lower in PCOS especially in women <40 years, and hs-CRP levels were increased in women with PCOS <30 years, but these differences disappeared after BMI-adjustment.
The three best predicting factors for PCOS were FAI (≥2.0, OR 8.18), A (≥9.7 nmol/l, OR 6.16) and T (≥1.1 nmol/l, OR 4.17).
Conclusion
The analyses performed with LC-MS confirm the results of earlier studies using RIAs or other older methods, showing that increased serum T levels in PCOS persist until menopause. The results also indicate that FAI and serum androgens serve as the best predictors of PCOS at all ages, while the prediction value of metabolic factors do not perform equally well as they may associate more with obesity than with PCOS *per se*.

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OC5.4

Influence of variants in the *FSHB* and *FSHR* gene on reproductive parameters in males and females

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Background
Recently, a single nucleotide polymorphism (SNP) in the *FSHB* promoter (−211G>T, rs10835638) was found to be associated with lower serum FSH levels and oligozoospermia in males. In contrast, a SNP in the FSH-receptor gene (FSHR, 2039A>G, rs6166) was previously shown to be associated with FSH levels in women only.
Subjects and methods
One thousand two hundred and thirteen male partners in infertile couples without known causes for male infertility and 365 thoroughly characterised women with normal menstrual cycle intervals and proven ovulation were genotyped for both SNPs by TaqMan assay. Associations between genotypes and clinical parameters were evaluated.
Results
In males, the *FSHB* −211G>T T-allele showed significant dosage effects for FSH (−0.51 U/l per T-allele), LH (0.28 U/l) and bi-testicular volume (−3.2 ml). In contrast, The *FSHR* 2039A>G G-allele exhibited non-significant trends for associations with higher FSH and reduced testicular volumes. However, in the combined model, *FSHR* 2039A>G significantly modulated the more dominant effect of *FSHB* −211G>T on serum FSH and testicular volume among the T-allele carriers.
In contrast, in women the *FSHB* −211G>T T-allele was associated with both higher FSH (0.99 U/l) and LH levels (1.30 U/l) and with reduced progesterone (−1.96 ng/ml). The *FSHR* 2039A>G G-allele was associated with higher FSH levels (0.35 U/l per G-allele). Numbers were too small to calculate combined SNP effects.
Conclusions
The SNPs in the *FSHB* and *FSHR* genes have significant impact on reproductive parameters in both sexes and the combinatory effects of variants in hormone and receptor are an unparalleled example in endocrinology. Gender specific mechanisms, probably involving progesterone in females, may explain the partially opposing findings concerning FSHB −211G>T. Oligozoospermic patients carrying unfavourable variants affecting FSH action may benefit from FSH treatment and women undergoing IVF may receive tailored ovarian stimulation in the future.

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OC5.5

FSHB −211 and *FSHR* 2039 polymorphisms are associated with serum levels of FSH, AMH and age of pubertal onset in 78 healthy girls: a longitudinal cohort study

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Context
Potency of the FSH-pathway varies according to polymorphisms in the promoter of the gene encoding the FSH beta subunit (*FSHB* −211 G>T, rs10835638) and in the gene encoding the FSH receptor (*FSHR* 2039 A>G, rs6166). A recent study suggested that carriers of the combination of *FSHB* GG and *FSHR* AA had the most reproductive 'fit' phenotype (higher serum FSH and a more sensitive FSH receptor). In pre-pubertal girls, FSH and AMH levels correlate negatively indicating a pituitary–gonadal set-point.
Objective
To evaluate if polymorphisms in *FSHB* and *FSHR* were associated with reproductive parameters in healthy girls.
Design and setting
Seventy-eight healthy girls were examined biannually in the COPENHAGEN Puberty Study; number of examinations per girl, median (range), 9 (2–13). Serum levels of hormones were measured by immunoassays. Genotyping was determined by PCR amplification followed by restriction enzymes specific for the genotypes (RFLP analysis).
Main outcome measures
Association analyses of single and combined polymorphisms with FSH, AMH, and age at pubertal onset.
Results
Carriers of (*FSHB* GG+*FSHR* AA) had higher levels of FSH prior to pubertal onset, lower mean serum levels of AMH, and they entered puberty earlier than girls with other combinations of genotypes; FSH 2.2 (1.3–3.9) vs 1.5 (0.4–4.4) IU/l ($P=0.05$), AMH 13.8 (5.6–53.9) vs 19.4 (4.8–47.4) pmol/l ($P=0.04$), and age of pubertal onset 9.7 (8.8–10.7) vs 10.6 (9.0–13.5) years ($P=0.03$), respectively.
Conclusions
Our findings indicate that genetic polymorphisms in the FSH pathway influence timing of puberty in healthy girls, as well as the pre-pubertal FSH-AMH set-point. Furthermore, it seems that individual AMH levels are determined by common variations in genes regulating follicle growth as well as the number of resting primordial follicles.

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OC5.6

INSL3 in 268 male patients with congenital hypogonadotropic hypogonadism (CHH): effects of different modalities of hormonal treatment

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Context
Insulin-like peptide three (INSL3) is a testicular hormone, secreted by Leydig cells, during fetal life, in newborns, and after puberty. These 3 phases of testicular activation are impaired in CHH.
Objective
To evaluate in a single center circulating INSL3 in a significant series of men with CHH in order to asses the consequences of pre- and postnatal gonadotropin deficiency, to specify the INSL3 regulation by gonadotropins and to evaluate the potential diagnostic interest of this peptide.
Patients
Two hundred and sixty-eight CHH patients (77 untreated, 97 receiving testosterone and 94 under combined gonadotropin therapy (hCG and FSH)) and 40 age-matched normal men were evaluated.
Methods
Serum INSL3 was immunoassayed by a validated sensitive and specific RIA.

Results
Mean INSL3 levels (±S.D.) were 827±364 pg/ml in normal men and dramatically decreased (61±42 pg/ml; $P<0.001$) in untreated CHH patients, all of whom had values below controls. Positive correlations between both serum total testosterone (T) or LH and INSL3 (respectively $P<0.01$ and $P<0.0001$) were observed in untreated CHH. In T treated CHH mean INSL3 was also very low (50±25 pg/ml) but in CHH receiving combined hCG and FSH therapy, INSL3 was very significantly higher (402±292 pg/ml; $P<0.001$) than in untreated CHH. Combined FSH-hCG therapy (from 61±21 to 301±254 pg/ml) or hCG alone (from 48±10 to 223±114 pg/ml) significantly and prospectively increased INSL3 levels in CHH, contrary to T (from 61±21 to 53±37 pg/ml) or FSH monotherapies (from 48±10 to 60±16 pg/ml).
Conclusions
The dramatic INSL3 decrease in CHH is caused by LH deficiency and correlated with its severity. The increase in INSL3 levels during combined gonadotropin and hCG monotherapy reinforces its exclusive Leydigian origin and its positive regulation by LH/hCG. Finally, INSL3 seems as sensitive as total testosterone to evaluate the testicular Leydig function in CHH and is therefore a useful diagnostic marker.

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Diabetes & Obesity

OC6.1

Type 2 diabetes risk alleles frequencies in the Portuguese population

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Introduction
Type 2 diabetes is one of the most common health problems worldwide and its prevalence is rapidly increasing. Although environmental factors play a substantial role in the etiology, genetic susceptibility has been established as an important risk factor. Several recent genome-wide studies and linkage analysis testing have identified and confirmed various T2D susceptibility *loci*. However, as demonstrated by the results of the HapMap project and by other studies, the allele frequencies of the risk variants differ between populations.
Material and methods
As a preliminary study of the genetic risk evaluation in Portuguese T2D patients, we selected 18 variants that have been consistently associated with T2D (PPARG, KCNJ11, TCF7L2, WFS1, KCNQ1, HNF1B, HHEX, NOTCH2, CDC123, TSPAN8, CDKL1, SLC30A8, CDKN2BAS, ADAMTS9, FTO, IGF2BP2, JAZF1, and THADA) and analyzed 571 DNA samples of a normal Portuguese population. The allele frequencies were calculated and compared with the published frequencies for other European populations.
Results
For all variants, genotype call rates were >99%. All except one variant (TSPAN8 rs7961581) were in Hardy–Weinberg equilibrium Compared with other studies including normal European Caucasian populations, the allele frequencies of this population were in the same range but still with some differences, more evident for variants in CDKL1, SLC30A8, ADAMTS9, TCFL2, HHEX, HNF1B and WFS1. All these variants are primarily related to β-cell function and affect insulin secretion.
Conclusion
These results indicate that the Portuguese population profile for these variants has its own identity and confirms the relevance of this study as a preliminary step for the evaluation of risk alleles in the Portuguese diabetic patients.

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OC6.2

Dissociation between high fat diet-induced obesity (DIO) and insulin resistance: lessons from AHNAK knockout mice

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Obesity is usually considered to be a forerunner to insulin resistance (IR). Using *in vitro* studies, we have identified a role for the giant protein AHNAK in regulating Glut4 expression and function. *Ex vivo* analysis of white adipose tissue from ob/ob mice and aged/obese rats exhibited elevated AHNAK protein levels (2.3- and 3- fold) with concomitant reduction in Glut4 levels (5- and 2-fold).
The aim of this study was to investigate the role of AHNAK in overall metabolic homeostasis using AHNAK knockout (KO) mice. When challenged with high fat-diet (HFD) for 12 weeks, AHNAK KO mice were protected from DIO as reflected by a 50% reduced adipose tissue mass, 25% reduced body weight and 37% increased lean body mass, compared to wild type (WT) mice on HFD. KO mice exhibited IR as evident from their glucose (AUC 28412 vs 34307) and insulin (AUC 12645 vs 15056) tolerance tests. Fasting blood glucose levels were 17% elevated while plasma insulin levels were 37% reduced in KO-HFD compared to WT-HFD. Contrary to the *in vitro* results, Glut4 levels in adipose tissue obtained from either KO-chow or KO-HFD were 35 and 20% reduced compared to WT-chow. Real-time PCR analysis showed no change in levels of adipogenic genes PPARgamma and CD36, while muscle and liver SCD-1 levels were 4-fold upregulated in KO. The expression of G-6-Pase, key gluconeogenetic enzyme, was higher in KO compared to WT mice.
Taken together, our data show dissociation between DIO and IR in AHNAK KO mice. AHNAK has an important role in weight control, but AHNAK deficiency impairs insulin sensitivity *in vivo*. Therefore, careful regulation of AHNAK is essential for the management of metabolic homeostasis.

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OC6.3

Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin–angiotensin–aldosterone system in healthy men

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Glucagon-like peptide-1 (GLP-1) is an incretine hormone with multiple actions besides control of glucose homeostasis. GLP-1 is known to cause natriuresis in humans but the effects on basic renal physiology are still partly unknown.
This study therefore aimed to demonstrate the effects of GLP-1 on kidney hemodynamics, electrolyte handling, and the renin–angiotensin–aldosterone system in healthy men.
Twelve healthy young males were examined in a randomized, controlled, double-blinded, single-day, cross-over trial to evaluate the effects of two hours GLP-1 infusion on kidney functions. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were assessed with ^{51}Cr-EDTA and ^{123}I-hippuran, respectively, using a constant infusion renal clearance technique based on timed urine sampling.
GLP-1 had no significant effect on either GFR (+1.9%, 95% CI (−0.8; 4.6%)) or RPF (+2.4%, 95% CI (−3.6; 8.8%)). Fractional urine excretion of lithium increased 9% ($P=0.013$) and renal sodium clearance increased 40% ($P=0.007$). Angiotensin II decreased 19% ($P=0.003$) while renin, aldosterone, and the urinary excretion of angiotensinogen showed no significant changes.
The results indicate that although GLP-1 markedly reduces proximal tubule sodium reabsorption, the acute effects on GFR and RPF are very limited in healthy humans. The finding of GLP-1's ability to reduce angiotensin II concentration is novel and may play a part in the possible kidney protective properties of GLP-1.

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OC6.4

Reduction of fat mass with the mTOR inhibitor, sirolimus, in humans: from transplantation to lipodystrophies

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Objectives
Sirolimus inhibits adipocyte differentiation (Yeh PNAS 1995). This study compares weight and fat markers in two groups of patients treated or not with sirolimus, before and after transplantation.
Patients and method
Nineteen islet-alone transplanted patients treated with sirolimus and 7 islet-alone or liver-transplanted patients NOT treated with sirolimus were compared 1 year after transplantation in terms of weight, fat mass (equation of body fat and percentage of fat mass by DEXA), and metabolic parameters. 14/19

islet-alone transplanted patients treated with sirolimus were reassessed 5 years post-transplantation.
Results
Before transplantation, the metabolic and weight/fat parameters were similar in the 2 groups except for C-peptide. One year post-transplantation, body fat and leptin reduction was more important in the sirolimus than in the NON-sirolimus group ($P<0.05$). Leptin levels were lower in the sirolimus group ($P=0.01$). Compared to pre-transplant values, one year post-transplantation, weight, fat mass and metabolic parameters did not change in the NON-sirolimus group while the sirolimus group showed a significant reduction in weight ($P<0.001$), BMI ($P<0.001$), body fat ($P<0.001$), percentage of body ($P<0.05$) and truncal ($P<0.05$) fat mass, HbA1c levels ($P<0.001$) and ßscore ($P<0.01$). Compared to pre-transplant values, the sirolimus group showed a significant reduction of leptin level one ($P=0.004$) and five years ($P=0.01$) post-transplantation, as well as a persistent reduction of HbA1c (before: 8.4 (1.5); 1-year: 5.8 (1.7); 5-years 6.7 (1.7)% and ß score (before: 0.0; 1-year: 7.0 (3.5); 5-years 4.0 (4.0)) ($P<0.05$). Sirolimus correlated with leptin ($r=-0.22$, $P=0.018$) and body fat mass ($r=-0.18$, $P=0.03$).
Conclusion
These results suggest that sirolimus modulates the amount and/or the quality of adipose tissue and innate immunity, opening new perspectives both in the choice of immuno-suppressant, and the treatment of nucleopathies, especially lipodystrophies (*Ramos Sci Transl Med* 2012).

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OC6.5 – ESE Young Investigator Award

The role of costimulatory molecules B7.1 and B7.2 in obesity-related adipose tissue inflammation and liver steatosis

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Introduction
Macrophages and lymphocytes are considered as major players in adipose tissue (AT) inflammation and liver steatosis, contributing to metabolic dysregulation during obesity. The costimulatory molecules B7.1 and B7.2 are important regulators of T-cell activation and inflammatory reactions, however, their contribution in obesity-related AT and liver low-grade inflammation is poorly characterized.
Methods
Mice sufficient or deficient in B7.1 and B7.2 (double knockouts, Dko) were fed a low or high-fat diet (HFD) for 18 weeks. Body weight was measured weekly and metabolic functions were assessed by measuring blood glucose, cholesterol, triglycerides, insulin, leptin, adiponectin and performing glucose and insulin tolerance tests. Subcutaneous and gonadal AT were excised and the stromal vascular fraction (SVF) was analyzed by FACS. Liver steatosis was evaluated by measuring intratissular triglycerides and confirmed by H&E and Oil-Red stainings. Liver non-parenchymal cells were isolated and analysed by FACS. The levels of inflammation, thermogenesis and fat deposition genes were measured by qPCR in white and brown adipose tissue and liver respectively.
Results
B7.1 and B7.2 expression was upregulated in gonadal AT and SVF upon HFD. Dko mice displayed higher glucose, cholesterol and leptin levels and worse GTT when fed a HFD compared to the B7.1/B7.2 sufficient mice. This was accompanied by downregulation of Tregs, CD4 and CD8 lymphocytes and a parallel upregulation of proinflammatory M1 macrophages (defined as F4/80+ CD11b+CD11c+ or F4/80+CD11c+CD206-cells) in both subcutaneous and gonadal AT. Dko mice showed also increased liver steatosis, linked with increased levels of inflammatory M1-like macrophages and reduced levels of Tregs in the liver. In accordance, Dko mice had higher levels of inflammation and fat accumulation genes in AT and liver respectively and lower levels of UCP-1 in the brown AT.
Conclusion
The B7.1-B7.2 signalling pathways are significant regulators of the obesity-related adipose tissue inflammation and liver steatosis pathophysiology.

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OC6.6

Serum- and glucocorticoid-regulated kinase 1 in obesity-related adipose tissue and peripheral inflammation

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see P728.

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Nurse Posters

N1

Pegvisomant home care program is likely to improve treatment compliance leading to rapid IGF1 control

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Background
Pegvisomant has demonstrated efficacy in attaining IGF1 normalisation in previously uncontrolled acromegalic patients. Improper or less than prescibed use may lead to suboptimal control.
Aim
Evaluation of home educational program.
Methods
Multicenter Flemish study in seven non-controlled acromegalic outpatients. All patients were trained for daily s.c. pegvisomant injection at home by one single specialist nurse during 2 h. Enhanced motivation was provided through regular follow-up visits on day 2, 3, and 7 (60′, and twice 45′, respectively) and 15′ phone calls at month 1, 1.5, 3, 4.5, 6, 12, and 18. Preliminary results include IGF1 at baseline, 3 and 4.5 months.
Results
Seven acromegalic patients uncontrolled with octreotide LAR ($n=4$; 30 mg monthly) or lanreotide ($n=3$; 120 mg per 3 weeks or monthly) were included in this observational study.
Upon dose adjustment from 10 OD to 20 mg OD for patient 1 IGF1 at 4.5 months further decreased to 152. For patients 2 and 5 IGF1 further decreased without dose titration to 253, and 107 respectively. No adverse events were recorded, except for more frequent hypoglycemic episodes in 1 insulin-treated woman with type 2 diabetes.

Table 1

	1	2	3	4	5	6	7
Age (gender)	61 (F)	59 (F)	43 (M)	62 (F)	64 (M)	66 (M)	62 (M)
Pegvisomant starting dose (mg)	10 OD	10 OD	10 OD	10 EOD	10 OD	10 OD	10 OD
IGF1 baseline (ng/ml)	347	1032	311	289	299	877	341
IGF1 3 months later (ng/ml)	326	335	157	93	124	280	50

M, male; F, female; OD, once daily; EOD, every other day.

Conclusion
In all participants IGF1 decreased significantly ($P=0.018$) within a time period of 3 months. Working with a specialist nurse may prove a key to success in attaining rapid IGF1 normalisation, due to enhance patients' compliance.

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N2

Psychiatric morbidity in pediatric patients after surgical remission of Cushing's disease: case presentations

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Context
Endogenous Cushing's syndrome (CS) may have different effects in children than what has been described in adults. Prior studies of children and adolescents with CS have identified cognitive decline despite reversal of brain atrophy after remission of CS, as well as residual impairment in quality of life measures. Although the observations of parents of children and adolescents with CS support personality changes, significant psychopathology has not been described in the literature.
Setting
Subjects were enrolled in a clinical protocol at the National Institutes of Health Clinical Center in Bethesda, MD, USA.
Clinical presentation
We report six children (4M, 12.4 ± 2.5 years), who underwent successful transsphenoidal surgery (TSS) for treatment of Cushing's disease (CD), and subsequently developed significant affective symptoms (range for onset of symptoms 4–60 months). Affective symptoms included: decline in school performance, anger/rage outbursts, suicidal ideation, anxiety, and depression. One child (18 years old female), who committed suicide 60 months post-TSS, had recently discontinued antidepressant medication. She had a history of anxiety during active CS and was treated with an anxiolytic. The 4 patients with onset of symptoms within 7 months of TSS were on glucocorticoid replacement, and 1 year follow-up evaluation showed recovery of HPA axis and biochemical evidence of remission. The two patients who presented with onset of symptoms 48 months or later, underwent endocrine evaluation that showed biochemical evidence of remission and normal anterior pituitary hormone levels.
Conclusion
A longitudinal study of adults reported an incidence of psychopathology of 66.7% in active CS that declined to 24.1% at 12-month after remission (Dorn 1997). This is the first report of affective symptomology/behavioral dysregulation, including suicidal ideation, in a subgroup of children and adolescents after remission of CS. Health care providers should screen for changes in symptomology, including suicidal ideation, anger outbursts, anxiety, and depression and refer to appropriate mental health professionals.

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N3

Living in the shadow and light: Iranian youths' response to diabetes-related stigma

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Introduction
Diabetes is one of the most acceptable chronic diseases. However, diabetes-related stigma is proposed as an important and striking phenomenon in many countries especially in Asian countries such as Iran. This study aimed to explore the responses of Iranian young people with type 1 diabetes to the diabetes-related stigma.
Methods
Conventional qualitative content analysis approach guided this inquiry. Volunteered people with type 1 diabetes were recruited by purposeful sampling from one endocrine and metabolism center in Isfahan in 2012. Data gathering was done through 17 individual unstructured in-depth interviews and three focus groups. Data saturation was achieved through 33 participants. The data were analyzed using qualitative content analysis.
Results
All participants acknowledged stigma and responded it in different ways, which was categorized in two main categories including living in the shadow (hide and seek, missing diabetes, withdrawal) and moving toward light (diabetes disclosure, destruction of the false bubbles).
Conclusion
The most response especially for girls was to live in the shadow of silence that can be associated with negative consequences affecting diabetes management. Moving towards the light, suggests that it is possible to help people with type 1 diabetes to achieve a normal life as much as possible. It is necessary to plan the anti-stigma programs and engage them actively to reduce stigma and mitigate or prevent its negative impacts.
Keywords
Diabetes-related Stigma, People with Type 1, IRAN.

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N4

Nurse led telephone clinic: antenatal hypothyroidism

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Background
Hypothyroidism is characterised by insufficient secretion of the thyroid and its detection measured by elevated serum TSH levels.
Although relatively common during pregnancy, untreated maternal hypothyroidism is associated with adverse obstetric and neonatal outcomes such as

preeclampsia, placental abruption and miscarriage, or foetal neurodevelopment defects.
Highlighting a nurse led specialised telephone clinic in the close monitoring and care of these women is vital.
Aims
To provide for pregnant women with hypothyroidism a specialised Nurse-led Clinic, with structured pathways, in collaboration with multidisciplinary teams, to ensure:

- Continuity of care with reduction in requirement for face-to-face appointments.
- A streamlined service supported by evidence based practice.
- Earlier detection of elevated TSH levels above 2.5 IU/l.
- Optimising treatment management.
- Providing support and education, enhancing adherence and reducing non-attendance.

Structure:

- Referral of women with history of hypothyroidism, or TSH levels above 2.50 IU/l.
- Medical history and results discussed with physician.
- Appointments booked within 2 weeks where condition education, support and treatment management are reviewed.
- Electronic recording of visit.
- On-going review throughout each trimester prior to discharge.

Caseload
Seventy-three new referrals were reviewed over a 3-month period, of those 30% within their first-trimester, and 44% in their second trimester presented, with elevated TSH levels above 2.5 IU/l on referral.
This reflects positive identification patients at risk, due to improved multi-disciplinary team communication facilitated by the specialist nurse.
Conclusion
Standardised protocols ensure earlier referral; resulting in the provision of high-quality patient focused Nurse-led care, treatment management and patient education of gestational hypothyroidism. Through a service systematically developed for continual improvements that are directly relayed to the patients, while providing a cost effective service for patients and service providers, while ensuring nursing activity is recorded and can be audited.

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N5

Assessing treatment satisfaction, knowledge and adherence of patients attending a pituitary Nurse-led Clinic

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Background and aim
Polypharmacy is characteristic of endocrine conditions and patients who are satisfied and knowledgeable of their treatment have better outcomes. The aim of this study was to measure treatment satisfaction, knowledge and adherence in patients with a pituitary condition attending a Nurse-led Clinic.
Methods, sample and data collection
Data were collected using a postal survey between January and March 2010 and included a total of 262 patients. The questionnaire which was developed for the purpose of this study includes two subscales; i) the 18-item Satisfaction and Knowledge subscale with a 5-point Likert scale 'strongly agree to strongly disagree' and ii) the 8-item Adherence subscale with a 5-point Likert scale 'never to always' and a 'not applicable' 6th point for patients taking their medication on a weekly/monthly basis. Statistical analysis was performed using SPSS version 19 for Windows.
Results
A response rate of 58% was achieved ($n=152$; 84 females and 68 males). Mean age was 42 years (S.D. = 15.7; range = 19–79). Mean duration of diagnosis was 16 years (S.D. = 11.8; range = 1–45). Patients were taking anything from 1 to 6 different medications for their endocrine condition (mean = 2.6; S.D. = 1.5) with almost a third taking between 4 and 6 medications. A high level of Satisfaction and Knowledge was reported (mean = 3.8 (S.D. = 0.7); items SUM = 64.6 (S.D. = 11.6) with scoring range 17–85). Similarly, patients reported high levels of Adherence to treatment (mean = 4.1 (S.D. = 0.8); items SUM = 20.3 (S.D. = 0.8) with scoring range 5–25).
Conclusion
The results of this audit emphasise the important role that the Endocrine Nurse plays in patient education and in achieving better treatment satisfaction and adherence.

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Poster Presentations

Adrenal cortex

P1

Chronocort®, a multiparticulate modified release hydrocortisone formulation, shows dose linearity and twice daily dosing provides physiological cortisol exposure

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Cortisol has a distinct circadian rhythm; levels rise from 0300 h to peak within an hour of waking and gradually decline until 1800 h before a quiescent period lasting from 1800 to 0300 h. Current hydrocortisone replacement regimens are unable to replicate this rhythm and we have been investigating modified release technology. Our initial formulation, using tableting technology, demonstrated it was possible to replicate the overnight rise in cortisol but the tablet had reduced bioavailability (Debono *et al*. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 1548–1554). We have now investigated the use of multiparticulate technology which has flexibility for variable doses using the same formulation. We tested six multiparticulate formulations and identified a formulation, DIURF-006, that provided the optimal overnight cortisol profile. We have now tested dose-proportionality and twice daily dosing for DIURF-006 using 5, 10, and 20 mg administered as a single-dose at night and 30 mg administered as a 20 mg dose at night and a 10 mg dose the following morning in 16 healthy male volunteers in whom endogenous cortisol levels were suppressed with dexamethasone. Dosing was randomised, all studies were separated by 1 week wash-out period and cortisol levels measured by LC–MS/MS. Serum cortisol levels were shown to increase linearly with dose, although dose proportionality was not at unity as shown in the slope of the regression line: C_{max} for 5, 10, 20, and 30 mg was 288, 440, 641, and 665 nmol/l (slope: 0.49, 95% CI 0.42–0.56) and AUC0-t was 1586, 2530, 3919, and 5610 h×nmol/l (slope: 0.69, 95% CI 0.65–0.73). The AUC and C_{max} for twice daily dosing were similar to that reported for normal diurnal cortisol secretion. In conclusion, Chronocort® formulation DIURF-006, provides a linear increase in C_{max} and cortisol AUC over a dose range from 5 to 30 mg and the C_{max} and AUC are similar to that previously reported for physiological cortisol levels.

Acknowledgements
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P2

Impaired quality of life in CAH adults is associated with adiposity and insulin resistance

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Background
Quality of life (QoL) has been variously reported as normal or impaired in congenital adrenal hyperplasia (CAH) adults. We found impaired QoL in UK CAH adults (Arlt *et al*. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 5110–5121) and now report the relationship between QoL (SF-36) and health outcomes in these patients.
Methods
Cross-sectional analysis of 151 CAH adults with 21-hydroxylase deficiency aged 18–69 years. QoL was transformed into age- (decade) and sex-adjusted *z*-scores using reference data from random sample of 14 430 UK subjects aged 18–79 years (Prof. John Brazier, Sheffield University). Principal components analysis (PCA) was undertaken to identify clusters of inter-related clinical and biochemical features (waist circumference, systolic and diastolic blood pressure, serum triglycerides, HDL-cholesterol, HOMA-IR testosterone, androstenedione, 17-hydroxyprogesterone and mutation severity). The principal component (PC) scores were used in multiple stepwise regression analysis as predictor variables of QoL.
Results
Three PCs were identified by PCA that explain 61% of the total variance (r^2) in observed variables. Regression analysis demonstrated that PC2, reflecting *adiposity and insulin resistance* (waist circumference, serum triglycerides, HOMA-IR and HDL-cholesterol), related to individual QoL scores, specifically impaired physical function ($\beta=-0.72$, 95% CI: -1.11 to -0.34, $r^2=19.9\%$, $P<0.001$), bodily pain ($\beta=-0.51$, 95% CI: -0.77 to -0.23, $r^2=21.6\%$, $P<0.001$), general health ($\beta=-0.50$, 95% CI: -0.80 to -0.20, $r^2=16.0\%$, $P=0.001$), vitality ($\beta=-0.44$, 95% CI: -0.65 to -0.16 $r^2=15.5\%$, $P=0.002$), and the Physical Components Summary Score ($\beta=-0.58$, 95% CI: -0.83 to -0.33, $r^2=26.4\%$, $P<0.001$), but not to the Mental Components Summary Score.
Conclusions
Increased adiposity and insulin resistance are associated with impaired QoL in adults with CAH. Intervention studies are now required to establish whether reduction in metabolic risk factors can improve QoL in this disadvantaged patient group.

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P3

Correlation between cell cycle, steroidogenesis and PKA RIA and RIIB subunits in adrenocortical tumors cells

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The cyclic AMP (cAMP) signalling cascade is one of the main pathways involved in the pathogenesis of adrenocortical tumors (ACT). *PRKARIA* or *PRKARIIb* are involved in the proliferation/apoptosis in a subset of tumors.
Majors alterations of genes involved in both cell proliferation and the cell cycle have been described by transcriptome and miRNome analysis in various types of adrenocortical tumors (ACC, ACA, AIMAH, and PPNAD). In addition to tumor growth for malignant tumors, steroid excess causes morbidity in patients with all types of ACT.
The goal of this study is to find whether there is a correlation between the cell cycle and the steroidogenesis.
Methods
We have studied the cell cycle distribution (FACS) and the expression of the steroidogenic enzymes (western blot, and RT-qPCR) in i) H295R cell line after inactivation of *PRKAR1A* or *PRKARIIb* using RNA interference ii) synchronized H295R cell line, and iii) PPNAD cells in primary cell culture. We used pharmacologic drugs to arrest cells at specific cell cycle check point: L-mimosine (G1 phase), aphidicolin (S phase), and nocodazole (G2 phase). The expression of the different actors of cell cycle regulation as cyclins and cdks, and signalling pathways were studied.
Results
The decrease of either R1α or R2β protein enhances the accumulation of cells in G2 phase, and Cyp17A, Cyp11B1 and Cyp11B2 levels. The synchronization of both the H295R (ACC cells) or PPNAD primary cell culture at G2 phase increased the expression of the steroidogenic enzymes. In PPNAD this increase started at the S phase. Arresting both H295R and PPNAD cells in G1 phase decreases the steroidogenic enzymes expression, resulting in a decrease in cortisol secretion.
Conclusion
We have found a correlation between the cell cycle check point and the expression of steroidogenic enzymes. Targeting specific cell cycle check point may down regulate the hyper secretion of steroids in these tumors.

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P4

Long-term follow-up in adrenal incidentalomas: an Italian Multicentre Study

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Introduction
The need of a long-term follow-up in adrenal incidentalomas (AI) is debated and data on cardiovascular events (CVE) are lacking.
Methods
In this retrospective study all patients referred to seven Italian Endocrine Centers for AI, without signs of hypercortisolism at baseline and with a $\geq$5 years follow-up (80.7$\pm$30 months, range 60–286), were enrolled. From 171 patients (121 F) aged 59.5$\pm$10.2 years (range 25–79) the changes in weight, glucose and lipid metabolism, blood pressure control and the occurrence of CVE were obtained. Patients were classified as affected with subclinical hypercortisolism (SH) in the presence of cortisol after 1-mg dexamethasone suppression (1-mg DST) test $>$5 μg/dl or $\geq$2 parameters out of low ACTH, increased urinary free cortisol and 1-mg DST $>$3 μg/dl.
Results
At baseline SH was found in the 14% of patients. The prevalence of obesity, diabetes mellitus, dyslipidemia, and arterial hypertension (54, 21, 25, and 45% respectively) was not different between patients with (SH+) and without SH (SH−). At baseline SH+ patients showed a higher CVE prevalence than SH− ones (21 vs 7%), regardless of age (OR 3.3, 95% CI 1.0–11.1, $P<0.05$). At the end of follow-up a new diagnosis of SH was made in the 7.6% of patients, whereas in the 3.5% the SH diagnosis was not confirmed. The adenoma size (baseline 2.3$\pm$0.8 cm) increased $>$2.5 cm in the 2.9% of cases. The glucose and lipid metabolism, blood pressure, and weight control worsened in the 24, 16, 36 and 14% of patients, respectively. The SH persistence/appearance was significantly associated with the worsening of $\geq$2 out of the metabolic parameters ($P=0.013$) and with the occurrence of new CVE ($P=0.05$).
Conclusion
In AI patients a long-term follow-up is recommended for the diameter increase and SH development risk. SH patients are at risk of worsening of the metabolic control and, importantly, of CVE.

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P5

The gene expression profile of cortisol secretion in adrenocortical adenomas

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The cortisol secretion level of adrenocortical adenomas range from hormonally silent to overt hypercortisolism. The mechanisms leading to the autonomous hypersecretion of cortisol are unknown. The aim was to identify the gene expression alterations associated with the autonomous and excessive cortisol secretion of adrenocortical adenomas.
Methods
The transcriptome of 22 unilateral adrenocortical adenomas (5 non-secreting, 6 subclinical cortisol-producing, and 11 cortisol-producing) was studied and correlated with cortisol secretion. Phosphodiesterase 8B (PDE8B) expression was measured by Western Blot.
Results
Unsupervised hierarchical clustering identifies two groups of adenomas with a difference in secretion level ($P=0.008$). Cluster 1 includes only cortisol-producing adenomas (8 out of 11), while Cluster 2 is an admixture of the non-secreting, the subclinical cortisol-secreting and 3 of the 11 cortisol-secreting adenomas (Fisher exact $P=0.002$). This cluster is driven by genes related to cortisol secretion and to extracellular matrix.
More than three thousand genes correlate with cortisol secretion. Among the positively correlated are the steroidogenic enzymes, genes involved in cholesterol metabolism and glutathione S-transferases. Among the negatively correlated genes are genes related to transcripts translation and the transcription factor GATA-6.
The PDE8B, which inactivates the PKA pathway, unexpectedly showed the strongest positive correlation with cortisol secretion, confirmed by Western Blot. The PKA-activity/cAMP ratio was increased in adenomas with high PDE8B levels, suggesting that the PDE8B increase is a counter-regulation to limit downstream activation of the pathway.
Conclusion
The transcriptome of adrenocortical adenomas shows a major association with cortisol secretion and identifies specific groups of genes implicated in steroid secretion. Among them, the cAMP/PKA pathway seem altered in cortisol secreting adenomas.

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P6

Activation of EGFR promotes ACC cell proliferation by inducing VEGF autocrine secretion

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Adrenocortical cancer (ACC) is a rare and aggressive malignancy. Currently the main therapeutic option is surgery, but due to difficult and delayed diagnosis and to the onset of metastases, medical therapy is often tried. ACC treatment is mainly represented by Mitotane alone or in association with chemotherapy, with variable results. Understanding the molecular mechanisms that regulate ACC proliferation could be useful to identify new therapeutic options. Aim of our study is to identify growth factors that may regulate ACC proliferation, using two human ACC cell lines, the SW13 and the NCI-H295 cells. Our data show that epidermal growth factor (EGF) and transforming growth factor (TGF)-α enhance SW13 cell proliferation and reduced apoptosis, while had modest effects on NCI-H295 cells. Sunitinib, an EGF receptor (EGFR) inhibitor, and NVP-BEZ235, a PI3K/mTOR inhibitor, reduced cell viability in both cell lines, being counteracted by both EGF and TGF-α in SW13 cells. Since in other settings EGF regulates cell proliferation by inducing VEGF, we investigated VEGF secretion by the two cells lines. EGF and TGF-α enhanced VEGF secretion only in SW13 cells while had no effects on NCI-H295. In addition, a VEGF receptor blocking antibody significantly reduced EGF and TGF-α induced cell proliferation.
We investigated in both cell lines the expression of EGFR, which is higher and ubiquitous in SW13 cells, while it is weaker and sparse in NCI-H295 cells, where it is present only on the membrane.
These data demonstrate that EGF and TGF-α are important in regulating Sw13 cell proliferation, also by modulating VEGF secretion. In conclusion our data suggest that EGF pathway could represent a new molecular target in drug design for treatment of ACC that display enhanced EGFR expression.

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P7

Human leukocyte antigen (DQ2/DQ8) and 21-hydroxylase antibodies determine the thyroid peroxidase antibody status of patients in autoimmune Addison's disease

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Autoimmune Addison's disease (AAD) results from the immune mediated selective destruction of adrenal steroid hormone-secreting cells. Autoantibodies (Abs) against 21-hydroxylase (21OH) are diagnostic present in 85–90% newly diagnosed patients. Its genetic susceptibility is conferred by human leukocyte antigen (HLA) DQ2 and DQ8. In many patients autoimmunity extends forming the autoimmune polyglandular syndrome type 2 (APS-2). The aim of this study was to test, whether specific HLA alleles in combination with the 21OHAb status were associated with thyroid autoimmunity as detected by thyroid peroxidase autoantibodies (TPO-Abs) in patients with AAD.
Patients with AAD ($n=194$) were genotyped for HLA. and TPO-Abs were measured using an enzyme-linked immunosorbent assay, 21OH-Abs with an *in vitro* transcription/translation method. The titers of Abs were quantified and defined as positive (pos) or negative (neg) (21OH-Ab $>48<$index; TPOAb $>100<$UI/ml). Statistical analysis used Kruskall-Wallis and Spearman's correlation tests.
HLA high risk (R) alleles (DQ2 and/or DQ8), $21OHAb^{pos}$ and $TPOAb^{pos}$ were present in 71, 86 and 36% of the AAD patients, respectively. Furthermore, in order to evaluate the effect of HLA/21OH-Abs status on the production of TPO-Abs the patients were divided into four subgroups (Gr): Gr1: $HLA^{high\text{-}R}$/$21OH\text{-}Ab^{neg}$; Gr 2: $HLA^{high\text{-}R}$/$21OH\text{-}Ab^{pos}$; Gr 3:$HLA^{low\text{-}R}$/$21OH\text{-}Ab^{neg}$; Gr4:$HLA^{low\text{-}R}$/$21OH\text{-}Ab^{pos}$. While the Gr 2 and Gr 4 had significantly higher concentration of TPO-Abs in median (44.5 and 55 UI/ml) than Gr 3 (1.3 UI/ml; $P_{corrected}=7.4\times10^{-4}$ and 7.4×10^{-3} respectively) no difference was observed compared to Gr 1. Also a significant correlation was found between 21OH-Abs and TPO-Abs titers ($\rho=0.21$, $P=0.01$).
Both the genetic background of AAD as detected by the HLA risk alleles DQ2/DQ8 and the status of the 21OH-Abs are associated with thyroid autoimmunity in APS-2. This combination may be an indicator of the enhanced risk of polyglandular destruction in autoimmune Addison's disease.

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P8

Plasma metanephrine for assessing the selectivity of adrenal venous sampling

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Context
More reliable parameters than cortisol are needed for assessing correct catheter positioning during adrenal vein sampling (AVS). Plasma metanephrine represents one such alternative.
Objective
To determine the utility of adrenal venous (AV) plasma concentrations of metanephrine to establish correct catheter positioning during AVS.
Design and methods
We included 86 AVS procedures: 52 ACTH-stimulated and 34 non-stimulated sequential procedures. Plasma cortisol, metanephrine, normetanephrine, epinephrine and norepinephrine concentrations were measured in AV and peripheral venous (PV) samples. AVS success rates, according to cortisol AV:PV selectivity indices of 2.0 and 3.0, were compared with that for metanephrine using a selectivity index (SI) determined by ROC curve analysis.
Results
Among AVS procedures assessed as selective using a cortisol SI of 3.0, the median AV:PV plasma metanephrine ratio was 6-fold higher than that for cortisol (94.0 vs 15.5, $P<0.0001$). There were significant positive relationships between AV:PV ratios for cortisol and metanephrine for ACTH-stimulated samplings, but not for non-stimulated samplings. ROC curve analysis indicated a plasma metanephrine SI cut-off of 10. There was 96% concordance in AVS success rates determined by cortisol and metanephrine derived SIs in ACTH-stimulated AVS. Without stimulation, the concordance was 82 and 56% for at respective SIs of 2.0 and 3.0; AVS success rates determined by metanephrine (91%) were higher than those determined by cortisol (56 and 79% for respective SIs of 3.0 and 2.0).
Conclusions
Metanephrine provides an alternative analyte to cortisol for sensitive assessment of AVS selectivity that appears particularly advantageous in sampling performed without ACTH stimulation.

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P9

Insulin sensitivity in patients with Addison's disease: a randomised cross-over trial comparing conventional glucocorticoid replacement therapy with continuous subcutaneous hydrocortisone infusion therapy.

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Background
Conventional glucocorticoid replacement therapies result in unphysiological variation in plasma cortisol levels; concern has been raised regarding long-term metabolic consequences. Glucocorticoid replacement is technically feasible by continuous subcutaneous hydrocortisone infusion (CSHI), which can mimic the normal diurnal cortisol rhythm. The aim of this study was to compare insulin sensitivity in patients with Addison's disease (AD) on CSHI vs three daily doses (t.i.d.) of oral hydrocortisone.
Design, subjects, and measurements
This was an open randomised, cross-over trial, comparing 2 months of CSHI vs t.i.d. oral hydrocortisone with a minimum of 3 months wash-out period in patients with long duration of AD. Treatment A was oral hydrocortisone with weight-adjusted doses as suggested by Mah *et al*. (1). Treatment B was CSHI using Solu-Cortef® (50 mg/ml) with a body surface area adjusted dose (10 mg/m^2 per 24 h). Insulin sensitivity (mg/kg per min) was determined after 2 month of each treatment using the euglycemic hyperinsulinemic clamp technique (40 mU/m^2). Whole-body insulin sensitivity (*M*-value) was calculated from the amount of glucose infused during the last 30 min of the clamp divided by body weight (kg) and period (min) and expressed as mg/kg per min.
Results
Fifteen patients (11 women and 4 men) age (49.4 ± 6.2 years) and AD duration (15.5 ± 10.3 years), took part in the study. After 2 months treatment (cross-over) the mean dose of oral hydrocortisone was (17 ± 2.5 mg) and Solu-Cortef® in CSHI was (19.6 ± 2.5 mg) respectively. There were no absolute differences between treatment A vs B regarding; BMI (24.8 ± 2.1 vs 25.0 ± 2.1 kg/m^2; $P=0.74$), waist–hip ratio (0.84 ± 0.1 vs 0.85 ± 0.1; $P=0.65$), systolic blood pressure (115.9 ± 12.7 vs 114 ± 12.0 mmHg; $P=0.67$), nor in insulin sensitivity (8.1 ± 3.6 vs 7.5 ± 2.7 mg/kg per min; $P=0.59$) but there was a significant difference in diastolic blood pressure (77.2 ± 5.2 vs 72.4 ± 5.5 mmHg; $P=0.02$).
Conclusion
This is the first study to compare insulin sensitivity in patients with AD on CSHI vs oral hydrocortisone. CSHI replacement was not superior in terms of insulin sensitivity compared to conventional oral glucocorticoid replacement.
Reference
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P10

First phase insulin secretion is impaired by aldosterone excess in primary aldosteronism

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Context
Primary aldosteronism (PA) represents the most frequent cause of a curable secondary arterial hypertension. Conflicting data have been published regarding the effect of aldosterone excess on glucose and lipid metabolism.
Objective
Our aim was to analyze insulin sensitivity and beta cell function in a cohort of PA patients. Prospective follow-up investigations were performed in a subgroup of patients before and after adrenalectomy to assess the metabolic outcome.
Design
Oral glucose tolerance test, combined intravenous glucose tolerance test (ivGTT) - hyperinsulinaemic-euglycaemic glucose clamp test and arginine test were carried out after a 12-h fasting period.
Patients
Twenty two consecutive PA patients with both unilateral ($n=14$) and bilateral ($n=8$) disease were recruited through the Munich center of the German Conn's Registry. The control group of EH patients ($n=11$) of corresponding age, gender

and BMI were recruited from our hypertension unit. A normotensive cohort ($n=11$) was recruited as control group.
Results
At baseline, first phase insulin reaction in ivGTT was significantly reduced in patients with PA as compared to normal controls (36.0 (24.0; 58.7) vs 90.1 (52.6; 143.8) μU/ml, $P=0.031$) and lower in comparison to EH without reaching statistical significance (53.2 (30.8; 73.3) μU/ml, $P=0.123$). The study was repeated 6 months afterunilateral adrenalectomy in 9 consecutive patients with APA.At this time point, blood pressure was normalized in the majority of patients while BMI remained unchanged (26.9 (25.5; 37.6) vs 27.5 (25.1; 35.6) kg/m^2, $P=0.401$).The first phase insulin reaction in response to glucose significantly increased at follow-up (from 36.0 (25.5; 58.7) to 48.5 (30.4; 95.2) μU/ml, $P=0.038$). In contrast, insulin sensitivity, insulin resistance and response to i.v. arginine did not differ before and after adrenalectomy.
Conclusion
These findings provide evidence that aldosterone excess has a direct negative effect on beta cell function in patients with PA. Accordingly, following adrenalectomy, early insulin secretion improves significantly in these patients.
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P11

Molecular screening for personalized treatment approach in advanced adrenocortical cancer

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Propose
To screen for the presence of putative targets for new treatments in a large cohort of advanced adrenocortical cancer (ACC)
Experimental design
In 40 adult stage III-IV ACC primary samples, we used comparative genomic hybridization (CGH) and hotspot gene sequencing (with Ion Torrent) to describe the presence of copy number abnormalities and mutations in more than 40 genes involved in cancer development and putative drug sensitivity (HER2; EGFR; BRAF; KRAS; PIK3CA etc.).
Results
The most frequent copy number alteration observed was the deletion of the tumor suppressor genes CDKN2A (four cases of 28 that generate informative profiles; 14.3%) and CDKN2B (3/28 cases 10.7%) both located in the region Chr.9p21. Lower level loss of the region Chr.9p21 were also frequently observed (7/28 cases 25%). The most frequent mutations were in the genes of TP53, ATM and CTNNB1 (6, 5, and 4 cases 15, 12.5, and 10%). Amplifications of FGFR1, FGF9, and FRS2 have been seen in three different subjects 7.5%. Other abnormalities were detected in single patients (BRCA1, PSME3; RPTOR; MYC; ABL1; PTK2; FLT3, MDM2; ERBB4, SMO; STK11; and GNAS). Same recurrent association of abnormalities were: deletion of CDKN2A and ATM mutation; TP53 and CTNNB1 mutation.
Conclusions
Drugs targeting cell cycle could represent nowadays the most relevant new therapeutic approach for patients with advanced ACC. FGFs pathway could be a potential target for treatment in a subset of ACC patients, while treatment with other targeted therapies could have a rational, based on the genomic alterations, only in selected patients.
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P12

Abstract withdrawn.

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P13

Detection of circulating tumor cells in adrenocortical carcinoma: a monocentric preliminary study

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Objective
Adrenocortical carcinoma (ACC) is a rare malignancy, whose prognosis is mainly dependent on the stage at diagnosis. The identification of disease-associated markers representing solid biomarkers for early diagnosis and drug monitoring is mandatory to improve survival rate and life quality of patients. CTC are tumor cells originating from primary tumor or metastases. The tumor-induced angiogenesis and invasion allow aggressive tumors to release CTC into blood stream before any detectable metastases are established. Therefore, CTC detection may have enormous potential of assisting malignancy diagnosis, estimating prognosis and monitoring the disease. The presence of CTC in ACC patients have never been investigated so far.
Design and methods
CTC analysis was performed in 14 ACC and 10 adrenocortical adenoma (ACA) patients. Blood samples obtained before ($n=3$ patients) and after ($n=10$ patients) surgery were filtered on Screencell devices (Screencell®), polycarbonate membranes with 8 μm pores which isolate CTC on size-base.
Results
CTC were isolated in all ACC but not in ACA samples. Immunocytochemistry on CTC, compared to the primary tumors, revealed positivity for adrenocortical markers, confirming the adrenocortical origin. When ACC patients were stratified in two classes according to the cut-off of the median value of the clinical parameters (tumor diameter, Ki67, and Weiss) or to the presence/absence of metastasis, a statistical significant difference was found in the number of CTC post-surgery only when diameter (CTC/ml mean$\pm$s.d.: 2.70$\pm$3.70 vs 0.59$\pm$0.67, $P=0.028$ for diameter ≥ 8 and <8 cm respectively) and metastatic stage (CTC/ml mean$\pm$s.d.: 3.91$\pm$4.83 vs 0.70$\pm$0.70, $P=0.031$, for stage$=4$ vs the others respectively) were considered.
Conclusions
Our findings provide the first evidence that circulating tumor cells (CTC) may represent a valid and useful marker to support diagnosis in adrenocortical tumor pathologies. Moreover, CTC seem to correlate with some clinical parameters. Although very preliminary, these results, which need confirmation in a larger series, suggest a potential use of this so called 'liquid biopsy' for prognosis and for non-invasive monitoring progression and response to treatments.
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P14

New diagnostic methods for primary aldosteronism with specific antibodies

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Background
Among patients diagnosed with primary aldosteronism (PA) due to an aldosterone producing adenoma (APA), about 10% fail to normalize aldosterone hypersecretion after unilateral adrenalectomy, and may instead have bilateral aldosterone producing hyperplasia. With routine histopathology it may be difficult to distinguish between APA and hyperplasia. We have recently reported the use of specific antibodies to identify the source of aldosterone excess in the removed adrenal (*Journal of Clinical Patholology*, in press). The clinical utility of the procedure has now been evaluated in an extended patient material.
Material and Methods
Between 1986 and 2010, 121 patients underwent adrenalectomy for PA. The cure rate was 88% and median follow-up 4 years. Immunohistochemistry with specific antibodies against the steroidogenic enzymes for aldosterone (CYP11B2) and cortisol (CYP11B1) synthesis was performed in addition to routine histopathology in, until now, a subset of 40 adrenals from both cured and uncured patients.
Results
With immunohistochemistry, the cured patients had typically positive staining for aldosterone synthesis in the adenoma, but not in cortical nodules. Interestingly, in two patients where routine histopathology suggested hyperplasia, it was possible to diagnose an APA as only one nodule showed immunoreactivity for aldosterone. Rarely, cured patients showed nodular hyperplasia, staining positively for aldosterone, which may represent unilateral or bilateral disease. Uncured patients showed nodular hyperplasia with positive staining for aldosterone synthesis. Three assumed APAs stained negatively for aldosterone production but positively for cortisol synthesis.
Discussion
Immunohistochemistry can help to localize aldosterone hypersecretion. This is important for the postoperative follow up as APAs are usually cured, while patients with hyperplasia need long term follow-up due to risk of recurrence.
Conclusion
Immunostaining with antibodies against CYP11B2 and CYP11B1 provides important information about the origin of aldosterone hypersecretion. If our results are confirmed, routine use of immunohistochemistry should be recommended.

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P15

In vivo and *in vitro* evidence supporting SSTR/mTOR pathway targeting in adrenocortical cancer

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Objective
In vitro data suggest that somatostatin receptors (SSTR) and mTOR pathways might represent reasonable targets for novel therapies, since are involved in growth of adrenocortical carcinoma (ACC). However, *in vitro* analysis of combination treatments, targeting both mTOR and SSTR as compared to mitotane, and detailed tissue typing mTOR/SSTR pathway are poorly explored in ACC.
Methods
This study was designed to characterize the tissue expression of mTOR/SSTR pathway-related molecules in a series of ACC and to investigate *in vitro* the effects on growth of SOM230, everolimus and mitotane in ACC cells. Immunohistochemistry for mTOR-related molecules and SSTR subtypes 1 to 5 was performed on a series of 58 ACC and correlated with clinical characteristics. H295R and SW13 ACC cells were incubated with mitotane, SOM230 and everolimus, either alone or in combination. Cell viability was determined by WST-1 method, and drug interactions were calculated using the combination index (CI). Modulation of mTOR and SSTR 1–5 genes was evaluated under treatments using real time PCR.
Results
Heterogeneous profiles of mTOR-related molecules and SSTR expression was observed in ACC samples, with no significant correlations among the different molecules investigated nor with clinical or pathological parameters. *In vitro*, everolimus determined cytotoxic effects in both ACC cells, with a synergistic effect combining everolimus with either mitotane or SOM230 and with no significant modulation of mTOR gene. By contrast, SOM230 was not effective when used alone in either H295R or SW13 cells, while in combination with mitotane showed an antagonistic effect in SW-13 cells and a synergistic effect in H295R cells (in these latter cells with a significant up-regulation of SSTR genes).
Conclusions
SSTR/mTOR-related molecules are heterogeneously expressed in ACC and multimodal targeting of these pathways – alone or in association with mitotane – might represent an effective treatment in ACC patients to be explored *in vivo*.

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P16

Mortality in long-term follow-up patients with progressively increased patterns of subclinical cortisol hypersecretion

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Background
Subclinical Cushing's syndrome (SCS) is defined as alterations in hypothalamic–pituitary–adrenal axis without classic signs or symptoms of glucocorticoid excess. This condition has been associated to increased risk of adverse metabolic and cardiovascular outcomes, independently of other potential risk factors. However, is still not known if this condition could lead to a higher mortality respect to non-secreting adrenal masses (NSA).
Aim
To evaluate the overall mortality of long-term follow-up patients with NSA, intermediate phenotypes of subclinical hypercortisolism (minor–ImP and major–IMP), and SCS.
Methods
This study involved 167 subjects. None of the patients underwent to surgical treatment of the adrenal mass. According to our previous study, the 1-mg dexamethasone suppression test (DST) was used as primary diagnostic tool, and patients were classified as below: 110 were defined NSA and 4 SCS, using the most stringent cut-off values (<50 nmol/l and >138 nmol/l respectively). Of the 53 patients with cortisol post-DST between 50 and 138 nmol/l, 29 were defined ImP and 24 IMP, using plasma ACTH and/or urinary free cortisol as additional diagnostic tools. Mean duration of follow-up was 8.8 years.
Results
Patients age was different in the four groups ($P=0{,}001$). Mortality analysis was then performed by Cox regression, using age as covariate. The overall unadjusted hazard ratios were 2.80, 3.82, and 7.70 in ImP ($P=0.015$), IMP ($P=0.003$), and SCS ($P=0.008$), respectively. The hazard ratios adjusted for age were 3.22 and 5.53 in IMP ($P=0.010$) and SCS ($P=0.026$), respectively, when compared to the NSA group. No significance was found for ImP patients after adjustment for age.
Conclusion
Increasing patterns of subclinical cortisol hypersecretion are associated with increased overall mortality, independently of the effect of age. Further analysis are needed to increase the study population with data from medium-term follow-up.

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P17

Role of adrenal vein sampling in primary aldosteronism: impact of different diagnostic criteria on subtype diagnosis

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In patients with primary aldosteronism (PA), adrenal vein sampling (AVS) is considered the gold standard to distinguish between unilateral and bilateral autonomous production of aldosterone, while diagnostic imaging tests by CT scan or MRI are often inconclusive for the diagnosis. To date agreement is lacking on the best criteria indicating successful cannulation and lateralization.
The aim of the study was to evaluate the impact of different diagnostic criteria for the successful cannulation and lateralization on subtype diagnosis and to compare the difference of the findings between adrenal CT scan and AVS.
Seventy-four patients with confirmed PA underwent AVS. The different diagnosis of PA subtypes reached using AVS data assessed by more permissive (type 1) and strict (type 2) criteria were compared. Al patients performed CT scan before AVS and imaging results were compared with results of AVSs (using both criteria).
Using type 1 criteria AVSs were successful in 86% of patients, and in only 64.5% using type 2 criteria. Type 1 criteria led to a higher rate of diagnosis of unilateral PA (85% of successful procedures) than type 2 (75%). There was considerable disparity in the diagnosis reached, with a concordance in only 45% of patients. In conclusion more permissive criteria for successful cannulation and lateralization on AVS can lead to incorrect diagnosis and accordingly to inappropriate treatment options. In the selected group of patients with successful AVS, CT findings correlated with AVSs findings in 58.5% of patients using type 1 criteria and in 47.5% using type 2 criteria. Finale diagnosis was based on histological results in 36 patients (49%) which underwent adrenalectomy based on AVSs findings. On

the basis of CT findings alone 17% of patients from the first group and 32.5% of patients of the second group probably would have been incorrectly bypassed as candidates for adrenalectomy. CT scanning lacks sensitivity and specificity and should be followed by AVS, which is the only reliable means of differentiating unilateral from bilateral PA and lateralizing APAs preoperatively. However, there are still controversies to be solved by large prospective studies on the criteria to adopt for defining the most appropriate cut off for both correct cannulation and lateralization.

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P18

The diagnosis of nonclassical congenital adrenal hyperplasia due to 21 hydroxylase deficiency in woman can be established by genetic testing or urine steroid profile analysis but not by ACTH stimulation test

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Backgroud
ACTH stimulation test is considered the basic diagnostic tool in the diagnosis of nonclassical congenital adrenal hyperplasia due to 21 hydroxylase deficiency (CAH). The cut off of 17OHP stimulation recommended for diagnosis is 10 ng/ml.
Aim
To assess whether the recommended threshold of 17OHP after ACTH stimulation confirms nonclassical CAH among woman with hyperandrogenism and elevated basal 17OHP level.
Material and Methods
Twenty-seven women age 18–38 years with hyperandrogenism and suspicion of nonclassical CAH based on basal 17-OHP level above 1.7 ng/ml. All patients were ACTH stimulation test performed. If the level of 17-OHP stimulation reached 10 ng/ml or more the genetic testing for CYP21A2 mutation and/or urine steroid profile analysis (GS/MS) was done to confirm the diagnosis.
Results
The median basal 17-OHP level was 4 ng/ml (1.8–14). The pick median 17-OHP level after ACTH stimulation was 11.29 ng/ml (2.97–30). Among eight patients with 17-OHP stimulation >10 ng/ml (11.26–30) two nonclassical CAH were confirmed (17-OHP basal level was 14 and 13.75 ng/ml, after ACTH stimulation 15 and 16 ng/ml respectively). The urine steroid profile analysis in the rest six patients didn't confirm the diagnosis of nonclassical CAH. Three of them had genetic testing performed and were diagnosed as heterozygotes.
Conclusions
The diagnosis of nonclassical CAH based on threshold 10 ng/ml of 17-OHP stimulation after ACTH can lead to false positive diagnosis of nonclassical CAH and often unnecessary glucocorticoid introduction. The definitive diagnosis can be established based on genetic testing or urine steroid profile.

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P19

Genetic analysis does not confirm NCCAH in almost half of the women who had received this diagnosis: preliminary results of an audit

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Introduction
Non-classical congenital adrenal hyperplasia (NCCAH) due to 21-hydroxylase deficiency is one of the most frequent autosomal recessive diseases. Genetic analysis performed for genetic counselling revealed a miscorrelation with the clinical diagnosis in several patients at our centre.
Aim
Confirm the genetic diagnosis of NCCAH in women attended for this condition.
Materials and methods
Consecutive patients attended at our centre are to be included. So far 26 patients have undergone medical record study collecting clinical, hormonal, and therapeutic information at diagnosis and follow-up into a standardized database. Analysis of the 21-hydroxylase gene has been performed through polymerase chain reaction, sequencing, and family genetic testing when possible.
Statistics
Descriptive analysis; data are expressed as percentages and medians(P25, P75).
Results
84.6% of the patients were index cases. Age at first symptom was 16 years (9.23) and the number of symptoms was 2 (1–3). The most common manifestations were hirsutism (66.7%), oligomenorrhea (50.0%), infertility (15.4%), acne (11.5%), and alopecia (11.5%). At diagnosis, basal 17-hydroxyprogesterone(17OHP) was available in 73% of patients (21.6 nmol/l (6.97, 41)) and ACTH stimulation for 17OHP in 23% (141.9 (52, 175.2)). Genetic analysis revealed that 11.5% of the patients had no mutations, 30.8% were heterozygous for a single mild mutation, 30.7% homozygous for a mild mutation, and 26.9% compound heterozygotes for one mild and one severe mutation. The most common mutation was mild Val281Leu. Globally 42.3% of the patients' genetic results did not confirm their clinical diagnosis.
Conclusion
In a substantial subset of patients in follow-up for NCCAH, hormonal work-up at diagnosis was inadequate. In almost half of the patients the genotype did not confirm the diagnosis. The diagnosis was confirmed in 81.3% of those with a sufficient hormonal diagnosis and in 20% of those with an insufficient one. These results compel us to reconsider the diagnostic and therapeutic requirements of these patients.

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P20

Investigation of β-catenin, N-cadherin, and E-cadherin expression in adrenocortical tumors

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Background
Adrenocortical tumors (ACT) are classified as adenomas (ACA) or carcinomas (ACC). β-Catenin constitutive activation is a frequent alteration in benign and malignant ACT. E-cadherin was discovered as a protein associated with β-catenin which plays a crucial role in cadherin-mediated cell adhesion. N-cadherin seems to be involved in the development of malignant ACT, but information regarding expression of N-cadherin or E-cadherin in ACT is very limited.
Aim
To evaluate the expression of N-cadherin, E-cadherin and β-catenin in ACT and in ACC cell line models (H295R and SW13).
Methods
We analyzed differential expression of β-catenin, N-cadherin and E-cadherin by immunohistochemistry and by quantitative Real time-PCR in 71 sporadic ACT. This study included eight normal adrenal cortex samples (NA), 24 ACC, 18 aldosteronomas (APA), 23 cortisol producing adenomas (CPA), and 6 non-secreting incidentalomas (NSA).
Results
Real-time PCR: compared with NA, β-catenin was over-expressed in 50% of ACC (12/24) and 51% of ACA (24/47); N-cadherin was down-regulated in 75% of ACC (18/24) and in 60% of ACA (28/47).
IHC: 47% of ACC (7/15) and 33% of ACA (11/33) presented increased cytoplasmic and/or nuclear β-catenin accumulation; furthermore 100% of ACC (15/15) presented down-expression of N-cadherin and 18 of 33 ACA (55%) were down-regulated. We did not find expression of E-cadherin in any ACT. Interestingly, Spearman analysis showed correlation between β-catenin and N-cadherin expression (ACC vs ACA).
Conclusion
Our preliminary data suggest that β-catenin overexpression together with the aberrant expression of N-cadherin may participate to progression of ACT.

Identification of these and other differentially expressed genes may enhance our understanding of the molecular biology of ACT development, and may contribute in creating new diagnostic and prognostic tools.

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P21

Bilateral inferior petrosal sinus sampling in cushing's syndrome: comparison between an old and a new technique in naples experience

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Introduction
Bilateral inferior petrosal sinus sampling (BIPSS) is the test that offers the highest diagnostic accuracy in the differential diagnosis between pituitary and ectopic Cushng's syndrome (CS). The aim of this study was to compare the diagnostic accuracy of BIPSS performed in the last six years, after the change in the technical procedure with that performed in the past period in Naples centre.
Patients and methods
Seventeen patients with CS (14 patients with pituitary and 3 with ectopic CS) followed-up in our institution between 2007 and 2012 were compared with 10 retrospective patients with pituitary-dependent CS subjected to BIPSS. The change in technical procedure was the use of 4-french (instead of 5-french) hydrophilic-coated vertebral catheters introduced into femoral veins using the Seldinger technique.
Results
In the ten historical patients BIPSS yielded 3 (30%) false-negative, together with a discordant result between baseline and post-CRH stimulation in one case, whereas side-to-side (S/S) gradient indicating a correct lateralization in nine patients (90%). In the 17 recent patients, no false-negative or false positive cases were observed, although in one case an IPS/periphery (P) gradient was found only at baseline evaluation. Moreover, a S/S ratio correctly indicated the lateralization of the lesion in 100% cases. The mean post-CRH IPS/P ($P<0.01$) gradient was significantly higher in recent than historical patients, although no significant difference was found in mean IPS/P and S/S gradient at baseline evaluation. Sensitivity, specificity and predictive value of BIPSS in the recent group was of 100%, whereas sensitivity and positive predictive values were 70 and 100% in the historical group.
Conclusions
In Naples experience the diagnostic value of BIPSS has been improved in the last years likely due to the introduction of new catheters, permitting to reach a diagnostic accuracy and a predictivity of 100% cases.

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P22

21-hydroxylase and interferon omega autoantibodies in Turner syndrome

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Introduction
An increased frequency of autoimmune diseases and an elevated incidence of autoantibodies have been observed in Turner syndrome (TS), but indirect immunofluorescence (IIF) has not been able to demonstrate autoantibodies against the adrenal cortex. We asked if the more sensitive radioimmunosorbant assay employing recombinant human 21-hydroxylase was able to identify autoantibodies against 21-hydroxylase, (21OH-Ab) in TS patients; 21-hydroxylase is the major adrenal cortex autoantigen in patients with autoimmune Addison's disease. Moreover, TS patients were tested for antibodies against interferon omega (IFNw-Ab), a marker for autoimmune polyendocrine syndrome 1 (APS 1) where autoimmune Addison's disease is one of the main components.
Methods
Blood samples from 144 karyotyped TS (11–62 years) were assayed 21OH-Ab and IFNw-Ab using *in vitro* transcribed and translated autoantigen. An index was calculated with a cut-off point of 57 and 200 for 21OH-Ab and IFNw-Ab respectively.
Results
Autoantibodies against 21-hydroxylase with low indices were present in 6 TS patients (4.2%); none had INF-omega autoantibodies. Overall, the TS patients had a mean age of 31.6 years (range 11.2–62.2). 53% ($n=77$) had the karyotype 45X. Hypothyroidism was recorded in 9% ($n=20$), coeliac disease in 1.4% ($n=2$), and type 1 diabetes mellitus in 0.7% ($n=1$). The six TS patients with 21-hydroxylase antibodies had a mean age of 32.7 years (range 17.7–44.7). Two had the karyotype 45X. One patient had hypothyroidism, but none had clinical apparent Addison's disease.
Conclusion
21-hydroxylase autoantibodies can be detected by using RIA in some patients with TS. These findings add to previous studies showing a high proportion of TS with an array of different autoimmune antibodies. Whether any of the autoantibody-positive TS patients will eventually develop Addison's disease remains to be seen.

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P23

Immunohistochemical markers of adrenal cortical tumors

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Introduction
Adrenocortical tumors (ACTs) are usually divided in adenoma (ACA) or carcinoma (ACC) according to histopathologic methods. Some lesions are occasionally difficult to classify according to these criteria. We studied the use of some immunohistochemical markers to recognise the difference between malignant and benign tumors.
Materials and methods
We studied 12 patients affected by ACC and 10 by ACA. Clinical evaluation and hormone analysis were performed in all patients who underwent to adrenalectomy. Immunohistochemisty was performed on adrenal tumours tissue except for a singular case in which materials come from lymph node metastasis. We analysed Ki-67, IGF2, Ghrelin, PPARγ, and ACTH expression.
Results
All ten ACAs showed a low Ki-67 $<5\%$, while 4 out of 12 (33%) ACCs showed a high proliferative index (Ki-67$>5\%$). The Wilcoxon–Mann-Whitney U test demonstrated difference between ACAs and ACCs for Ki-67 ($P>0.025$). We didn't find statistically significant differences between IGF2 ($P<0.0462$), Ghrelin ($P<0.738$), PPARγ ($P<0.403$), and ACTH ($P<0.369$). Although there were not differences for IGF2 between the two groups, we observed an overexpression of this marker in 50% of ACC (IGF2$>60\%$). Analysis based on Spearman correlation didn't find correlation between stage disease, tumor dimension and immunohistochemical markers in ACCs.
Conclusion
According to the literature, we found Ki-67 could be able to distinguish between ACAs and ACCs. Although many studies considered IGF2 as a malignant parameter, our results didn't confirm its use alone could be helpful to identify malignant lesions. Besides we showed the other different immunohistochemical markers, less commonly investigated in these tumors, should not be useful to discriminate adenoma from carcinoma. None immunohistochemical marker considered in our study showed a correlation with the characteristics of size or local extension of lesions. Therefore, we need more studies about a higher number of patients to obtain much more significant data about IHC utility.

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P24

Management of Addison's disease patients using dual release hydrocortisone during periods of intercurrent illness

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Introduction
Current management of intercurrent illness (ICI) in adrenal insufficiency (AI) is inadequate. We attempted to optimise ICI regimens with pharmacokinetic simulations of a dual-release (DR) hydrocortisone (HC) and tested them in a formal clinical trial.
Methods
This work consisted of: i) dosing recommendations in episodes of mild ICI with DR-HC using simulations with a population pharmacokinetic model, ii) collection of ICI episodes with increased cortisol use and outcome assessment using quality-of-life (QoL; Fatigue Impact Scale, Short-Form 36), and iii) exposure-response model for probability of developing an ICI based on logistic regression.
Data was collected from a 3-month cross-over trial comparing once-daily DR-HC with immediate-release (IR) HC given TID using the same daily dose of HC. Further safety data for DR-HC were available for 80 patients over 27 months.
Results
Cortisol profiles were obtained in 62 patients after administration of DR-HC on 116 occasions, and in 16 healthy volunteers. Simulations showed that an additional DR-HC dose at 8 ± 2 h after the morning dose or TID with 8 h intervals provided greater 24 h coverage IR-HC TID.
There was no difference in ICI episodes between DR-HC and IR-HC in mean percentage of days with extra doses, 2.5 and 1.6% respectively (median both 0.0). QoL after an ICI episode was not different between DR-HC and IR-HC. The total experience with DR-HC in mild ICI corresponded to 145 patient years (293 episodes).
There was no correlation between total HC exposure ($AUC_{0-24\ h}$) and numbers of AEs The estimated probability of at least one adverse event episode was not associated with total ($AUC_{0-24\ h}$) HC exposure; DR-HC ($P=0.64$) and IR-HC (TID) ($P=0.52$).
Conclusions
In this first prospective study in ICI, the suggested DR-HC regimens were successfully implemented in the clinical management of AI patients. Simulations suggested DR-HC regimens gave greater coverage over 24 h than IR-HC.

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P25

Abstract withdrawn.

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P26

Longitudinal assessment of adrenocortical responses to low-dose ACTH in critically ill septic patients

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Introduction
The hypothalamo-pituitary adrenal axis has been extensively investigated in sepsis. Most studies concentrated in the acute phase and in most the high-dose ACTH stimulation test has been applied. Studies extending in the post-acute phase by using the more physiological low-dose ACTH stimulation are scarce.
We aimed to investigate the time course of cortisol levels before and after stimulation with 1μg Synacthen in mechanically ventilated septic patients, over a 30-day period.
Methods/design
We studied 51 patients admitted to ICU with sepsis ($n=16$), severe sepsis ($N=19$), or septic shock ($N=16$). Total serum cortisol was measured before and 30-min after the i.v. administration of 1 μg Synacthen upon admission and every 3–4 days thereafter until administration of glucocorticoids ($n=6$), death ($N=15$), recovery ($n=24$) or completion of 30 days ($n=6$). Mixed-effect models were used to analyze the time progression of cortisol levels. Patients who received glucocorticoids were excluded from any analysis referring to survival.
Results
Administration of 1 μg Synacthen significantly increased cortisol levels at all time points ($P<0.001$). In the whole cohort, there was no significant variation in baseline and stimulated cortisol levels during the entire observation period. Throughout the study period patients admitted with septic shock had significantly higher baseline and stimulated cortisol levels compared to those admitted with sepsis or severe sepsis ($P=0.01$) and non-survivors had higher baseline cortisol levels compared to survivors ($P<0.001$). On admission, stimulated cortisol levels were similar between survivors and non-survivors but the difference between stimulated and baseline cortisol (ΔF) was lower in non-survivors. Subsequently, non-survivors had higher stimulated cortisol levels compared to survivors ($P=0.001$) with no difference in ΔF.
Conclusion
Baseline and stimulated cortisol levels remain relatively unchanged during the process of sepsis. Patients admitted with septic shock had higher baseline and stimulated cortisol levels, reflecting the severity of sepsis. Higher cortisol levels were associated with increased mortality.

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P27

Increasing prevalence of Addison's disease in Germany: health insurance data 2007–2011

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There are no epidemiological data of Addison's disease in Germany. Since there is no national registry for this rare disease alternative data sources need to be retrieved. The Technikerkrankenkasse (TK) is one of the large German health care insurance providers covering nearly 10% of the population. Documentation of diagnoses by all members with a doctor contact is evaluated by a scientific institute (WINEG). Screening of the database for Addison's disease was designed to filter out all causes of secondary, iatrogenic, traumatic or other non-idiopathic forms of primary adrenal failure. The prevalence of Addison's disease was found to range between 100 and 129 per million and showed a steady increase over the five years 2007–2011 of 22.5%. The prevalence was lower in men (73–90 per million, increase 18.9%) than in women (129–169 per million, increase 23.7%). Autoimmune comorbidities were found in 58.4%. Prevalence data for the more frequent autoimmune diseases type 1 diabetes mellitus and vitiligo did not change significantly over the years 2008–2011. These data provide a first epidemiological profile of this rare endocrine disease in Germany. Although the prevalence of Addison's disease appears slightly lower than in the Scandinavian countries, the increasing figures particularly in females warrant further investigation into causes and triggers of autoimmune adrenal destruction. Hereby we can show that health insurance data provide a valuable tool for epidemiological studies in the absence of national registries and allow providers to improve resource allocation and management of rare and also more common chronic diseases.

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P28

The role of late-night salivary cortisol measurement in the diagnosis of subclinical hypercortisolism in patients with adrenal incidentalomas

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The diagnosis of subclinical hypercortisolism (SH) is a challenging issue, especially as the high prevalence of adrenal incidentalomas (AI) is increasingly recognized. The diagnosis of subclinical hypercortisolism relies on a combination of tests that include overnight (ODST) or low dose dexamethasone suppression (LDDST), plasma ACTH, and 24-h urinary free cortisol (UFC). Late night salivary cortisol (LNSC) has been successfully used in the diagnosis of Cushing's syndrome (CS) and is now considered a first-line screening test. Nevertheless, its use in SH has been evaluated only in a limited number of reports. Therefore, we tested whether measurement of LNSC can be used to screen patients with AI for SH.

Thirty five patients (21 females) were studied; six patients had CS (group A); and 29 patients had AI. Based on published diagnostic criteria for SH (post-LDDST cortisol levels >1.8 μg/dl and at least one of the following: ACTH levels <10 pg/ml and high UFC >120 μg/24 h, corresponding to the upper normal limit of our assay patients with AI were divided in two groups: patients with SH (group B, $n=10$) and patients without SH (group C, $n=19$). Results were compared to normal controls (group D, $n=14$). LNSC was measured at 23.00 on the day of the study.

LNSC levels (μg/dl) were higher ($P<0.05$) in groups A (0.79 ± 0.36) and B (0.21 ± 0.06) compared to groups C (0.06 ± 0.01) and D (0.05 ± 0.01). ROC curve analysis revealed that SH can be diagnosed with the sole measurement of late-night salivary cortisol with 100% sensitivity but 50% specificity, with a cut-off of 0.045 μg/dl (AUC = 0.9 (95% CI: 0.79–1.03, $P=0.0008$). A higher cut-off of 0.055 μg/dl diagnosed SH with 90% sensitivity and 71.5% specificity. These data illustrate that late-night salivary cortisol is of value in the diagnosis of SH in patients with AI. Taking into consideration the ease of sampling, we suggest that salivary cortisol may be used as an additional screening test in this patient group.

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P29

Aldosterone measurement: performances of a new fully automated chemilunescence-based immunoassay

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Introduction

Measurement of aldosterone, the major mineralocorticoid secreted by adrenal cortex, is important for the screening of primary aldosteronism and for the clinical assessment of hypertensive patients. RIA remain widely used for the measurement of aldosterone but some automated immunoassays are emerging. The aim of our study was to evaluate the performances of a new automated aldosterone immunoassay.

Methods

Method imprecision of the Liaison® aldosterone assay (Diasorin), a fully automated immunoassay with chemiluminescence based detection, was determined with quality control materials and a pool of serum samples. Method comparison was performed with a commercial RIA (coat-a-count®, Siemens) in 91 patients samples.

Results

With quality control materials, between-run coefficients of variation (CV) were 8.3% at 4.9 ng/ml ($n=15$) and 5.3% at 25.4 ng/ml ($n=11$) with the Liaison® assay. For the serum pool with a concentration of 16.5 ng/ml, close to our laboratory cut-point of 14 ng/ml, the between-run coefficient of variation was 6.3% ($n=8$). A positive and significant correlation was observed between the two methods ($r=0.96$, $P<0.001$). For samples with aldosterone concentrations below 14 ng/ml ($n=65$), Passing and Bablock regression analysis provides a slope of 0.99 and an intercept of 0.02, without significant deviation of linearity. The mean difference observed on the Bland an Altman plot was 0.2 ng/ml. For samples with aldosterone concentrations higher than 14 ng/ml ($n=23$), Passing and Bablock regression analysis provides a slope of 1.00 and an intercept of -1.03, without significant deviation of linearity. The mean difference observed on Bland an Altman plot was 1.1 ng/ml.

Conclusions

Our study demonstrated satisfactory precision for the Liaison® aldosterone assay and results comparable to those of the RIA. Therefore, if our preliminary results are confirmed by larger studies, the Liaison® assay might be relevant for screening of primary aldosteronism and for the assessment of hypertensive patients.

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P30

Patients with Addison's disease have increased frequency of the metabolic syndrome: a case–control study

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Background

Patient's with Addison's disease (AD) have increased morbidity and mortality. Cardiovascular disease is considered the leading cause of death. The aim was to study cardiovascular risk factors in patients with AD.

Methods

In a single-centre, case–ontrol study 78 patients (52 women) with AD were compared to 78 controls, matched for gender, age, BMI, and smoking habits. Serum was collected in a fasting state for measurement of lipids and glucose metabolism, supine blood pressure measured and computed tomography used to assess abdominal subcutaneous and visceral fat. The IDF 2006 criteria were used for the classification of the metabolic syndrome (MS).

Results

Patients and controls were well matched with a mean age of 53 years and BMI of 25 kg/m^2. The mean (S.D.) duration of AD was 17 years (12). The median (range) daily hydrocortisone and fludrocortisone doses were 30 mg (10–50 mg) and 0.1 mg (0–0.2 mg) respectively. Sixteen patients (21%) with a mean age of 59.8 (10.5) years fulfilled the criteria for the MS compared to eight controls (10%, $P=0.09$) aged 69.7 (11.6) years.

Fifteen percent of the patients had diabetes mellitus (type 1, 6% and type 2, 9%) and 44% had hypothyroidism compared to 1 and 3% of the controls. More patients had pharmacological treatment for hypertension (22 vs 5%; $P=0.002$) and dyslipidemia (14 vs 1%; $P=0.003$).

The HOMA-index and the amount of abdominal subcutaneous and visceral fat mass were not different among the groups but triglyceride concentration was increased ($P=0.014$), LDL-cholesterol reduced ($P=0.025$) and HDL-cholesterol was increased ($P=0.091$) in the patients as compared with controls.

Conclusion

Patients with AD receiving standard replacement therapy have higher frequency of MS in a younger age and higher triglycerides compared to well matched controls. This may be explained by the relatively high hydrocortisone dose used for replacement. The control group which might contribute to the increased cardiovascular death previously suggested.

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P31

Reliability of serum versus salivary cortisol in ACTH test

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Introduction

Salivary cortisol measurement, representing free cortisol, seems to be a promissing alternative method to serum cortisol. Besides its use in the diagnostics of hypercortisolism, it could be of help in evaluation of adrenocortical reserve. It might be useful especially in patients on estrogen replacement, which modulates transcortin and hence total cortisol levels.

Aims

Compare the reliability of salivary vs. serum cortisol assessment during ACTH test.

Patients

We have performed ACTH test in 84 subjects (mean age 63.2; 66 men) with clinical suspicion on hypocorticism. According to the peak serum cortisol (≥ 500 nmol/l) patients were divided into two groups. Group A with normal response ($n=76$; mean age 64; 60 men) and group B with hypocorticism ($n=8$; mean age 61.4; six men).

Methods
250 μg teracosactide was injected intravenously. The blood and saliva were obtained before, in 30 and 60 min afterwards.
Results
Medians of serum cortisol in group A were 445; 766 and 902 nmo/l in 0, 30, and 60 min and 256; 394; and 453 in group B respectively. Medians of salivary cortisol were 6.9; 14.5 and 22.5 nmol/l in group A and 3.5; 5.1 and 4.8 nmol/l in group B. There was a significant correlation between serum and salivary cortisol levels during the test. The percent of variability as an expression of discriminating power, explained by the repeated measures ANOVA model, was significantly ($P=0.021$) higher for serum cortisol ($R^2=93.4\%$) compared to salivary one ($R^2=89.3\%$).
Conclusion
Our data show significant correlation between salivary and serum cortisol levels during the ACTH test. Measurement of serum cortisol has superior discriminating power compared to salivary cortisol in standard evaluation of adrenocortical reserve not influenced by estrogen replacement.

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P32

Diagnostic performance of first line biochemical tests to differentiate ACTH-ectopic syndrome among ACTH dependent Cushing's syndrome

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Introduction
This study evaluates the diagnostic accuracy of clinical features and first line routine screening tests to differentiate ACTH-ectopic syndrome from Cushing's disease (CD).
Materials and methods
The retrospective clinical and biochemical presentations of 180 patients with histologically proven ACTH-dependent Cushing's syndrome (CS) (159 CD, 21 ACTH-ectopic syndrome) were compared according to the cause of hypercortisolism. ROC-analysis was performed to estimate the diagnostic accuracy of the first line tests (2300 h serum cortisol, 24 h urinary free cortisol (24 h UFC) and ACTH rhythm in plasma) to differentiate ACTH-ectopic syndrome. A threshold for the test with the highest area under the curves (AUC) was chosen based on the maximum sum of sensitivity and specificity. Serum cortisol and plasma ACTH were assayed by electrochemiluminescence Cobas e601 Roche. 24 h UFC was measured by an immunochemiluminescence assay (extraction with diethyl ether) on a Vitros ECi
Results
The patients with ACTH-ectopic syndrome (in 15 cases bronchial carcinoid, in four – carcinoid of thymus, in one instance small cell carcinoma of the lung and in one – carcinoid of the appendix) had higher rates of low traumatic fractures ($P=0.04$), increased serum late-night cortisol, 24 h UFC, morning and evening ACTH and lower levels of potassium ($P<0.01$ for all parameters) vs CD. Late-night plasma ACTH showed the highest AUC (0.845 (95% CI 0.764–0.926)) to differentiate ACTH-ectopic syndrome from CD vs morning plasma ACTH – 0.790 (95% CI 0.673–0.908); late-night serum cortisol – 0.754 (95% CI 0.622–0.886) or 24 h UFC 0.619 (95% CI 0.481–0.758). A cut off value of 108.9 pg/ml for late-night ACTH yielded a sensitivity of 70.6% and a specificity of 81.7%.
Conclusions
Of all the clinical features and first line tests, the disturbance of the ACTH rhythm and high late-night plasma ACTH values in patients with proven CS is the most suggestive of ACTH-ectopic syndrome.

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P33

Salivary cortisol is a useful tool to assess the immediate response to pasireotide in patients with Cushing's disease

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Introduction
Pasireotide is a promising treatment option for patients with Cushing's disease (CD). The measurement of salivary cortisol is useful for diagnosing hypercortisolism and monitoring patients with CD following pituitary surgery. It may also be a better index of cortisol secretion than serum cortisol or urinary free cortisol (UFC). We investigated the value of salivary cortisol in monitoring short-term efficacy of pasireotide in patients with CD.
Methods
Seven patients (five females, two males; mean age 35.3 ± 7.4 years) received pasireotide 600 μg bid for 15 days in the Phase II study CSOM230B2208. Morning and midnight salivary cortisol, ACTH and morning serum cortisol were assessed at baseline and after 1, 5, 12, and 15 days of treatment. UFC was determined at baseline and day 15.
Results
On day 15, morning salivary cortisol had decreased in all patients; overall mean decrease from baseline was 70% (27.7 ± 30.8–8.2 ± 7.7 mmol/l). Midnight salivary cortisol had decreased in six patients and normalized in two; overall mean reduction from baseline was 50% (27.2 ± 38.6–13.4 ± 15.4 mmol/l). Decreases in morning and midnight salivary cortisol were observed from day 1 (mean reduction from baseline of 34 and 20% respectively) and persisted until day 15; the greatest decrease was on day 5 (mean reduction of 70 and 58%, respectively). At day 15, mean UFC had decreased from baseline by 65% (1711 ± 1941–593 ± 360 mmol/24 h). UFC was normalized in one patient (14%), who also had normalized midnight salivary cortisol, thereby restoring cortisol rhythm. Changes in ACTH and serum cortisol were similar to those of salivary cortisol.
Conclusions
Pasireotide rapidly reduced and normalized salivary cortisol. Salivary cortisol may be a simple, non-invasive biomarker to assess immediate response to pasireotide in patients with CD, particularly to determine whether cortisol rhythm is normalized in patients with normalized UFC levels. More studies are necessary to confirm these preliminary results.

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P34

Male hypogonadism in Addison's disease – an under-recognized problem

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Introduction
Male hypogonadism may complicate Addison's disease (AD), but the prevalence of testosterone deficiency in adult males with primary hypoadrenalism is unknown.
Methods
Male patients older than 18 years of age enrolled in the South African AD national registry were screened for biochemical testosterone deficiency (early morning basal testosterone <9.9 nmol/l). Testing was also performed to see if these subjects were more susceptible to metabolic disease.
Results
Of the 42 males studied, 14 (33%) were hypogonadal (5 previously diagnosed, 9 newly diagnosed). The presence of testosterone deficiency was not related to age, the duration of disease, or the hydrocortisone dose required. Underlying causes of AD for the hypogonadal group were autoimmune in 7 (50%), tuberculosis in 3 (21%), X-linked adrenal hypoplasia in 2 (14%) and 2 (14%) were idiopathic. None of the 14 hypogonadal subjects had anti-gonadal autoantibodies. Untreated hypogonadal subjects had a higher BMI compared to eugonadal subjects (29.4 kg/m^2 interquartile range (IQR): 24.8–32.5 vs 24.3 kg/m^2 IQR: 22.6–26.7, $P=0.029$), and a higher high-sensitive CRP (5.0 mgl/l IQR: 2.5–14.0 vs 1.5 mgl/l IQR: 0.6–2.8, $p=0.001$). There was no difference found between the two groups in terms of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides or fasting glucose. Luteinizing hormone and follicle stimulating hormone did not differ between the groups, however dehydroepiandrosterone sulphate was significantly decreased in the hypogonadal group (0.31 μmol/l, IQR: 0.27–0.37 vs 0.75 μmol/l, IQR: 0.51–1.50, $P=0.005$).
Conclusions
Biochemical testosterone deficiency was highly prevalent in this AD group and not related to age or duration of AD. Untreated hypogonadal subjects had an increased BMI and hsCRP, but no difference was found in their lipid profiles, or glucose levels. It may be worthwhile to evaluate all male patients periodically with AD for testosterone deficiency, as testosterone replacement may improve long-term subjective and clinical parameters.

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P35

A national survey on the prevalence and treatment outcome of active Cushing's disease in Belgium

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To estimate the point prevalence of active Cushing's disease (CD) in Belgium, all endocrinologists were invited to perform a retrospective chart review of the CD patients they had been treating between 1-1-2009 and 31-12-2010.
Only patients requiring cortisol lowering therapy because of cortisol excess could be included, such as de novo patients ($n=53$), previously diagnosed patients with persisting/recurrent disease after pituitary surgery and/or radiotherapy ($n=27$) or on primary or no medical therapy ($n=13$) and finally patients with previous surgical remission but relapsing in 2009 or 2010 ($n=9$). Other CD patients in remission or controlled without cortisol lowering therapy, e.g. those with bilateral adrenalectomy, were excluded.
The mean age at time of diagnosis of these 102 subjects (82% female) was 45 ± 15 yrs. The annual incidence of CD based on the de novo cases in 2009 and 2010 was 2.14 (95%CI: (1.36; 3.21)) and 2.77 (1.87; 3.95) per million inhabitants, respectively.
Over the two years period 57 pituitary surgeries were reported in 53 patients, followed by surgical remission in 79%. Remission rate (combined early remission and delayed control) was 72% in 46 first procedures and 82% in 11 repeat surgeries.
At the end of the observation period, 33 of 49 patients diagnosed before 2009 were still active. One had died while 15 were in remission after first (7) or repeat (7) pituitary surgery or after adrenalectomy (1). Of the newly diagnosed patients 30 were in surgical remission (following pituitary surgery in 26 and adrenalectomy in 4). After exclusion of another death, 22 new patients still had active CD on 31-12-2010, although in 13 of these, first surgical treatment had yet to be performed.
These data result in an estimated point prevalence of active CD on 31-12-2010 of 5.0 per million (95%CI (3.7; 6.5)), based on the entire Belgian population on that date.
This retrospective epidemiological study provides insight in the number of CD patients that might need additional treatment, including steroidogenesis inhibitors or pituitary-targeted drugs, when (repeat) pituitary surgery fails.
Could be candidates for treatment with the new pituitary-targeted drug pasireotide.
○ Audits should describe the patient population, audit methodology, and outcomes which should encompass any change/benefit to clinical practice.

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P36

More than two successive measurements of late-night salivary cortisol are needed to accurately diagnose early-stage post-surgical recurrence of Cushing's disease

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The performance of late-night salivary cortisol (LNSC) and optimal number of samples to accurately diagnose post-operative recurrence of Cushing's disease (CD) at an early-stage are unknown. We performed a retrospective analysis in a single tertiary-care center to compare the accuracy of multiple salivary sampling strategies to diagnose early-stage recurrences of CD. 36 patients in remission of CD after surgery were followed up for 69.2 ± 10.6 mo with multiple successive measurements of LNSC as part of long-term follow-up evaluation. Following an extensive biochemical evaluation, patients were classified as being in remission or in early-stage recurrence. The diagnostic accuracy of three diagnostic strategies combining 2, 3 or 4 LNSC was estimated and compared using areas under the ROC curves (AUC), sensitivity, specificity and predictive values. 44 sequences of assays (recurrence prevalence 52.3%) were available in 36 patients. The intra-sequence variability of LNSC was higher during recurrence (medians of S.D.s: 2.1 vs 0.5 nmol/l; $P<0.0001$, Wilcoxon test). AUCs ranged from 0.93 to 0.96 depending on the strategy. For 90% sensitivity, the best specificity (90.9%) was achieved when taking into account the maximum LNSC value in the four-first dosages strategy. For 25% recurrence prevalence, the proportion of false-negatives would be 3.7% and of false-positive 23.4%.
Conclusion
Due to a major within-patient variability of LNSC from one day to another, a strategy using 4 samples collected on successive days may detect early-stage recurrence of CD with a high accuracy, avoiding unnecessary hospitalization for complex testing in more than 75% of patients.

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P37

Adrenal venous sampling is mandatory and CT scan is unreliable for diagnosing unilateral primary hyperaldosteronism

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Introduction
There is ongoing debate about how to diagnose and treat unilateral primary hyperaldosteronism (PA). The use of CT scans and adrenal venous sampling (AVS) for the diagnosis of unilateral PA leading to unilateral adrenalectomy is still a matter of discussion. Moreover, some have suggested subtotal adrenalectomy when a detectable nodule is found on imaging studies while others prefer total adrenalectomy. We gathered information on imaging, diagnosis and treatment in Icelandic PA subjects.
Methods
A retrospective chart review was performed of all patients (age 18 and older) diagnosed with PA during 2007–2011 at the Landspitali University Hospital in Iceland, a referral center for the whole country (population of 318.000). All patients were diagnosed using the same standardized methods. After pharmacological modification, screening and verification testing, with salt loading and positional tests, all patients where further examined with a CT scan and AVS. When AVS indicated unilateral disease, patients were offered a laparoscopic total adrenalectomy.
Results
Of the 33 patients diagnosed with PA, 17 patients had bilateral disease and 16 patients unilateral. All 16 patients with unilateral disease had an adrenalectomy. Histopathology (HP) found 11 patients with cortical adenomas and 4 with hyperplasia. HP was inconclusive in one patient. Three patients with bilateral disease had a unilateral nodule on CT scans. Three of the patients with an adenoma did not have a nodule on a CT scan. One of the patients that had unilateral hyperplasia had a nodule on a CT scan. In the case that HP was inconclusive a nodule was described on a CT scan.
Conclusion
Adrenal venous sampling is mandatory in diagnosing unilateral PA and CT is not only unreliable but can be misleading in that matter. These results indicate that a total adrenalectomy is a favorable treatment option for unilateral PA.

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P38

Endosonography-guided fine-needle aspiration biopsy in differential diagnosis of adrenal gland tumours – pilot study

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Introduction
Adrenal incidentaloma is a problem with increasing importance. There are CT-density and washout criteria for distinction between adrenal adenoma and other tumours, there are well established protocol of screening for malignancy and/or hormonal dysfunction. But there remains small portion of atypical adenoma where detailed diagnostic is needed. One relatively new option is endosonography-guided fine-needle aspiration biopsy.
Aim
To assess validity, complications and diagnostic contribution of endosonography-guided fine-needle aspiration biopsy in patients with atypical adrenal tumour.
Methods
Pilot study – 14 patients with atypical adrenal mass were selected for the study. In all patients was performed common screening for malignancy and/or hormonal dysfunction. Excluding criteria was typical adrenal adenoma and functional

adrenal tumour. In all patients were examined plasmatic metanephrines and was excluded feochromocytoma before biopsy. All patients were admitted after endosnographic biopsy to clinical department and were observed till next day.
Results
14 patients, 6 men and 8 women were included in the study. In all patients were not observed any complication of biopsy. In 5 cases there were bilateral tumours, in 3 cases right adrenal tumours and in 6 cases left adrenal tumour. 3 tumours were bigger than 4 cm; others were smaller than 4 cm. In one case of unilateral tumour no biopsy was performed – adrenal gland was too small for secure biopsy; in one case of bilateral tumour was performed only one sample from anatomical reasons. In other cases totally 17 samples were taken; 2 were marginally sufficient, 15 were sufficient. In 9 cases, two bilateral and 7 unilateral, were biopsies benign and patients were observed. In 5 cases was indicated unilateral adrenalectomy. One feochromocytoma, one metastasis of ovarian carcinoid tumour, two adenocarcinoma and one adrenal cortex tumour (borderline characteristic between carcinoma and adenoma).
Conclusion
Endosonography-guided fine-needle aspiration biopsy seems to be useful and safe method of differential diagnosis of atypical adrenal tumours. It can reveal any primary or secondary malignancy in early stage, with good possibility of surgical treatment.

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P39

The Urinary Aldosterone in the Diagnosis of Primary Aldosteronism

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Introduction
The estimation of urinary aldosterone is one of the recommended confirmatory tests when diagnosing primary aldosteronism (PA). The study assessed the interpretation of the urinary aldosterone secretion (UA).
Subjects and methods:
The study enrolled both healthy volunteers and patients with suspected PA. Estimation of UA in 24-hour urine specimen was performed in enrolled individuals. Increased oral salt intake was recommended before and during urine collection. The data of healthy volunteers were intended to facilitate the interpretation of the data of patients with suspected PA. In patients with suspected PA, saline infusion testing (SIT) was performed twice in order to definitely confirm or exclude PA diagnosis. Both SITs were performed under identical conditions, i.e. after the drugs interfering with renin-angiotensin-aldosterone system were withdrawn. The individuals who exhibited discordant SIT results were excluded from the study.
Results
Based on the data of 30 healthy volunteers, upper UA reference value was determined: 43 nmol/day when urinary sodium exceeded 200 mmol/day.
Eighty-five urine samples from 45 patients with suspected PA were used to analyze the UA interpretation. In respect to PA diagnosis based on SIT, UA $>$ 19 nmol/day was characterized by 96% sensitivity and UA $>$ 92 nmol/day was associated with 96% specificity. Upper reference value based on the data from healthy volunteers was characterized by 77% sensitivity and 60% specificity.
Conclusions
Urinary aldosterone can reliably confirm or exclude the diagnosis of primary aldosteronism. However, this approach is applicable only in a limited number of individuals who exhibit very low or very high urinary aldosterone secretion.

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P40

Autoimmune polyglandular syndrome on a cohort of patients with primary adrenocortical insufficiency

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Introduction
On average, 40 to 50% of the patients with autoimmune adrenocortical insufficiency will eventually develop an autoimmune polyglandular syndrome (APS). Our aim was to characterize a population with primary adrenocortical insufficiency (AI) and determine the prevalence of other autoimmune disorders that might establish the diagnosis of APS.
Materials and methods
We included patients with primary AI under surveillance at our Department. Those with iatrogenic causes for AI were excluded. The referred population was characterised on what concerns to gender, age, disease's duration, familial history of AI and aetiology. We assessed autoimmune markers for Addison disease, type 1 diabetes, gastritis, thyroiditis and celiac disease in those without an obvious cause for AI. Positive markers triggered evaluation of associated dysfunction.
Results
Thirty patients (63.3% females) were included. Mean age- 46.9 ± 15.9; disease's duration- 19.9 ± 12.1 years. Familial history of AI was present in 23.3% of the cohort. On what concerns to AI aetiology, 63.3% were autoimmune, 20% congenital adrenal hyperplasia, 6.7% X-linked adrenoleukodystrophy, 3.3% tuberculosis, 3.3% antiphospholipid antibody syndrome and 3.3% undetermined. Considering the subgroup of autoimmune AI, 31.5% had at least one positive diabetes autoantibody (40% already diagnosed as having diabetes), 42.1% displayed autoimmune gastritis markers (62.5% with hypergastrinemia, 25% iron deficiency and 12.5% anaemia). Positive thyroid autoantibodies were present in 63.2% of the patients; 75% of them had thyroid dysfunction (hypothyroidism 88.9%). Celiac disease markers were not detected in this population.
Enough criteria for an APS diagnosis were fulfilled in 73.7% patients (85.7% APS type 2; 14.3% APS type 4).
Conclusion
The main aetiology for AI was autoimmune. The commonest associated autoimmune disorders were thyroiditis (63.2%) and gastritis (42.1%).
An early identification of other autoimmune disorders in patients with autoimmune AI will ensure an adequate treatment and follow-up, improving their quality of life. Therefore, a regular screening for autoimmunity is advisable.

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P41

Non-classic adrenal hyperplasia (NCAH) in patients with bilateral adrenal incidentally discovered tumors

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The prevalence of adrenal incidentalomas in computed tomography (CT) studies ranges from 0.6–1.9%. Adrenal masses are detected bilaterally in 10–15% of the cases.
Non-classic adrenal hyperplasia (NCAH), also termed as late onset of CAH, is a very mild form of 21-hydroxylase deficiency. The incidence of disease is estimated at 0.1% of population. Reported prevalence in women with androgen excess range from 0.6 to 9%.
Some patients have an overresponsive glucocorticoid response to ACTH stimulation, possibly reflective of subtle adrenal hyperplasia.
The aim of the study was to evaluate the prevalence of NCAH among patients with incidentally discovered bilateral adrenal tumors.
Material and methods
Seventy eight patients, 18 men and 60 women aged from 42 to 74 years with incidentally discovered in computed tomography (CT) bilateral adrenal tumors were examined. The diameter of tumors ranged from 17 to 52 millimeter and features of CT scanning suggested their benign character. Excess of the glucocorticoids or mineralocorticoids was excluded. Patients were asymptomatic or presented hardly mild symptoms (in women hirsutism and/or menstrual dysfunction). In 41 persons, 10 men and 31 women basal and ACTH-stimulated 17-hydroxyprogesterone (17-HP) concentrations were measured. A diagnosis of 21-OH-deficient NCAH was considered in patients with the basal 17-HP elevated or with the stimulated 17-HP level more than or equal to 10 ng/ml.
Results
In 19 patients (46.3%) – 4 men and 15 women NCAH was diagnosed. Fourteen patients had both elevated basal and stimulated 17-hydroxyprogesterone, while in five cases only elevation of stimulated level was found.
Conclusions
i). Non-classic congenital adrenal hyperplasia is a common autosomal recessive disorder and in case of incidentally discovered bilateral adrenal hypertrophy NCAH should be taken into consideration. ii). A basal 17-HP level is a useful screening tool for NCAH. iii). ACTH stimulation tests are essential to make proper diagnosis in some cases.
Keywords
incidentaloma, NCAH, 17-hydroxyprogesterone, ACTH.

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P42

Effect of glucocorticoids treatment on anthropometric parameters and sexual maturation rating in salt wasting and simple virilizing forms of congenital adrenal hyperplasia in Pakistani boys

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Congenital adrenal hyperplasia (CAH), caused by lack of 21-hydroxylase, impairs cortisol secretion, which increases ACTH release that results in hyperplasia of adrenal glands and increased secretion of adrenal androgens. Elevated androgen concentration leads to increased skeletal maturation, early pubertal development and diminished pubertal growth. Treatment with glucocorticoids averts early puberty but may abruptly slow down growth. The effect of exogenous glucocorticoids on adrenal and kidney functions, anthropometric parameters and sexual maturation rating was examined. Based on chronological age, twenty three (14 salt wasting (SW) and 9 simple virilizing (SV) types) male CAH patients were divided into group-I (2 patients of <1 year), group-II (4 patients of 1–2 years), group-III (15 patients of 3–8 year) and group-IV (2 patients of 9–11 years). SW types were treated with hydrocortisone twice a day (20 mg in the morning and 10 mg at night) along with a single dose of florine F (0.1 mg), whereas SV patients were treated with hydrocortisone twice a day (20 mg in the morning and 10 mg at night). CAH patients were compared with normal age matched controls (20 boys/group) before and following treatment. Regardless of age group and type of CAH, 17-OH P concentrations significantly decreased, concentrations of creatinine and urea were normalized but rennin activity remained unaffected in all patients. Concentrations of sodium and potassium were normalized in SW form, whereas cortisol concentrations were higher in SV and depressed in SW type. Concentrations of LH remained low but those of T were higher in most patients. Height and weight were normalized, whereas bone age remained advanced in all patients. Penile length remained larger, testicular volume was higher and pubic hair was advanced in all patients. In conclusion, appropriate glucocorticoids doses restored adrenal and kidney functions, normalized most of anthropometric parameters but did not affect sexual maturation rating.

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P43

Overt and ubclinical hypercortisolism in patients with diabetes mellitus and obesity

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Background
It has been hypothesized that patients with type 2 diabetes mellitus (DP) are very suspicious to have hypercortisolism, especially those which a high HbA1c level. The prevalence of hypercortisolism also could be high in obese patients (OP).
Methods
To 111 DP (male:female, 1:3; 58 (50; 64) years old; HbA1c, $9.5 \pm 2.2\%$), and 39 OP (male:female 2:1, 22 (20; 28) years old) 1 mg dexamethasone suppression test (DST-1) have been done. If post-DST-1 cortisol level was >50 nmol/l (positive result), the 2 mg/day for 48 h dexamethasone suppression test (DST-2) was made. If cortisol level exceeded 50 nmol/l after DST-2 (positive), subclinical hypercortisolism (SH) was suspect, and MRI, MSCT were done.
Results
Positive results was found in 39% of OP after DST-1 and reduced to 10% after DST-2. Only in one case (25%) abnormality was found (pituitary adenoma). In DP positive results was found in 42% of cases after DST-1 and reduced to 11% after DST-2. Among DP with positive DST-2 we incidentally revealed two patients with overt hypercortisolism (confirmed thereafter). Most (75%) of the DP and OP with SH had post-DST-2 cortisol level between 50 and 140 nmol/l. At the same time two patients with overt hypercortisolism had post-DST-2 cortisol level >140 nmol/l. There were no differences between HbA1c level in DP with negative and positive DST-1 and DST-2 ($P=0.3$). We made seven groups of DP depending on their HbA1c level. We did not find any differences in percent of patients with SH between groups. UFC and morning serum cortisol levels were higher in DP than in OP ($P<0.05$). Because DP were significantly older than OP ($P<0.05$), the two groups matched on age and BMI were created. Only the morning serum cortisol level was still higher on DP ($P=0.04$).
Conclusion
Diabetic patients are highly suspicious on subclinical and also overt hypercortisolism. In some of them even overt hypercortisolism can be missed. Obese patients also had high percentage of SH, but the group was small. The cut-off level of post-DST-2 cortisol 140 nmol/l can miss a lot of patients with SH.

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P44

Association of a variant of V281L of 21-hydroxylase gene CYP21A2 with simple virilizing and salt wasting types of CAH in Pakistani population

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused by deficiency of 21-hydroxylase (CYP21), which inhibits adrenal synthesis of cortisol and aldosterone. The reduced cortisol synthesis leads to increased ACTH secretion, which results in adrenal gland hyperplasia, accumulation of steroid precursors and excessive adrenal androgen production, causing pseudoprecocious puberty. The study aimed at determining variance in two SNPs, I172N and V281L, of CYP21A2 gene in twenty male CAH (13 salt wasting and 7 simple virilizing) patients and 20 age-matched controls. Genomic DNA was extracted and PCR-RFLP analysis was done. The genotypic frequency of wild type for two SNPs in case of controls and patients were 0.9, 1, 0.86, and 0.63% respectively. The heterozygous frequencies were 0.1, 0, 0.14, and 0.16% respectively. The PCR product of CYP21A2 I172N digested by Hind III gave bands of AA genotype comprised of a segment of $\sim$168 bp. The frequency of this genotype was 0.9% in controls and 0.86% in patients. Similarly, the genotypic frequency of other genotype AT, comprising segments of 168, 142, and 26 bp was 0.1% in controls and 0.14% in cases. The genotypes did not differ significantly between the two groups. Hence, the variant I172N of CYP21A2 was not associated with CAH. For V281L of CYP21A2 gene, PCR amplification yielded a segment of $\sim$207 bp. BamH1 gave bands of three different genotypes, CC (178 and 29 bp segments) in ten controls and 12 patients, AC (207,178, and 29 bp segments) in no control and three CAH patients and AA (segment of 207 bp) genotypes in no control and four CAH patients. In conclusion, V281L mutation was identified in seven of the total 20 patients studied. The frequency of V281L was 35% in CAH patients. The mutation V281L was found to be associated with both simple virilizing (20%) and salt wasting (15%) types of CAH.

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P45

Long-term efficacy of pasireotide in a patient with Cushing's disease and diabetes: results in the short term are not always predictive of long-term response

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The management of Cushing's disease (CD) can be problematic, particularly when the disease persists following pituitary surgery. Here we report the case of a 55-year old woman with CD that persisted after repeat transsphenoidal surgery. The patient had an overt phenotype with facial rubeosis, central obesity with supraclavicular fat accumulation, cervical fat pad and proximal muscle weakness. She had received anti-hypertensive drugs and insulin for diabetes mellitus.

Hormonal evaluations confirmed active hypercortisolism with absent cortisol rhythm, elevated midnight plasma cortisol (15.9 μg/dl) and increased 24-h urinary free cortisol (UFC > 5 × ULN) levels. After fulfilling inclusion criteria, the patient was randomized to pasireotide 900 μg b.i.d. in the Phase III study CSOM230B2305. During the first 3 months of treatment, UFC levels decreased from baseline by almost 50%. This was associated with improved clinical appearance (reduced facial rubeosis and supraclavicular fat accumulation; weight decrease of 7%), although worsening of diabetes was also observed. However, UFC levels remained elevated (2.5 × ULN) and the patient was considered to be a non-responder, therefore pasireotide dose was up-titrated to 1200 μg bid. From month 6 the patient had a progressive clinical and biochemical improvement, with resolution of hypertension, improvement of diabetes mellitus (with discontinuation of insulin and introduction of an oral hypoglycemic), and remission of all typical features of CD. Overall, her weight decreased by ∼30% from baseline. The patients' quality-of-life significantly improved, as did her sense of well being. At month 12, UFC levels were normalized and cortisol rhythm restored. Pasireotide dose was progressively reduced to 300 μg b.i.d. After 3 years the patient is still receiving pasireotide 300 μg b.i.d. with no loss of efficacy. In conclusion, short-term results are not always predictive of long-term response. In addition, pre-existing diabetes is not a contraindication to pasireotide treatment because the control of CD may outweigh any negative effects on glucose metabolism.

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P46

Autonomous aldosterone secretion as a significant cause of arterial hypertension: the effectiveness of targeted therapy

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Introduction
The prevalence of autonomous aldosterone secretion (AAS) in hypertensive subjects varies due to the use of various suppression tests and cut-off values. Our aim was to evaluate the prevalence of AAS in hypertensive subjects and test the anti-hypertensive effect of targeted treatment of the renin angiotensin II aldosterone system (RAAS) in AAS subjects.
Description of methods/design
We investigated 336 hypertensive subjects with and without adrenal adenoma and 83 age- and sex- matched normotensive subjects, with normal adrenal glands on computerized tomography. Serum aldosterone (ALD) and active renin levels (REN) and the aldosterone/active renin (ALD/REN) ratio were measured before and after a modified fludrocortisone suppression test (FDST - fludrocortisone suppression test after dexamethasone administration). Normal cut-offs were obtained from the controls (ALD levels post-FDST 82 pmol/l and ALD/REN post-FDST 25 pmol/mIU per l).
Results
By applying the combination of serum ALD post-FDST levels and the ALD/REN post- FDST ratio, the prevalence of AAS in hypertensive subjects was 31.3%. We found positive correlations between systolic (SBP) and diastolic (DBP) blood pressure and serum ALD and the ALD/REN ratio before and post-FDST ($P < 0.001$). After dividing in quintiles the ALD post-FDST levels of hypertensive subjects, the association of ALD post-FDST levels with SBP and DBP became statistically significant in the 4th (ALD levels > 101 pmol/l) and 5th quintile (ALD levels > 155 pmol/l) respectively. An inverse association between serum K^+ and ALD post- FDST levels was shown, that became significant for K^+ levels over 4 mEq/l.
Treatment targeting the RAAS with mineralocorticoid receptor antagonists or angiotensin II receptor blockers in 68 AAS subjects resulted in a marked decrease of SBP and DBP values ($P < 0.001$).
Conclusions
Using this methodology for detection of AAS an increased prevalence of AAS was found in hypertensive subjects. Targeted treatment of AAS subjects adequately controlled their blood pressure.

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P47

Cushing'S syndome: source of ectopic secretion of acth found after 20 years of follow-up

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A 59-year-old lady presented with Cushing's syndrome in 1991. Hormone testing revealed that it was due to ectopic secretion of ACTH. However, even after a comprehensive diagnostic workup, the source of ACTH secretion was not found. Since the symptoms (arterial hypertension and diabetes) were not successfully controlled with drugs, bilateral adrenalectomy was performed and the patient was put on hydrocortisone and fludrocortisone. The patient was then followed annually in the outpatient clinic. After 15 years of follow up, the ACTH levels started to rise: at first gradually and then duplicated in the year 2011. Morphologic diagnostic procedures, which became more accurate and easily accessible in the meantime, were repeated then. CT of the thorax revealed a 4 cm big tumorous formation in the left lower lung lobe. Unfortunately, we were not able to get a representative biopsy sample with bronchoscopy. The lesion was intermediate metabolically active on PET–CT and was positive for somatostatin receptors on octreoscan. Both studies were negative for possible secondary lesions in other parts of the body. The tumour was successfully removed with video assisted thoracic surgery and histologically confirmed to be a carcinoid. The cells of the tumor stained positive for ACTH. The control serum levels of ACTH were suppressed and stayed suppressed until now. In conclusion, we report a case of ectopic ACTH secreting tumour that was found after 20 years of follow-up and then successfully removed. Two things are important for planning care in the future and focusing possible research in such patients: i) since many tumours are small at least in the early stages, more accurate diagnostic methods provide a greater chance to find them. They should be repeated periodically (at least every few years). ii) It is important to efficiently control the cortisol excess, until the tumour is found.

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P48

Glucose tolerance in Cushing's disease

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Cushing disease (CD) is a rare affection due to an ACTH secreting pituitary adenoma with a hypercorticism as main consequence.
The aim of this study was to assess glucose tolerance anomalies in CD.
Patients
Twenty-one patients with a diagnosed CD were included; The majority of them (77.3%; $n = 17$) were female. Mean age was 34.2 (19–54 years) and mean BMI 31.9 kg/m^2 (25.9–43.7). Mean progression period of CD was 21.8 months (5–72). Catabolic signs and hypertension were present in respectively 85.7% ($n = 18$) and 68% ($n = 15$) of cases. Mean average of basal plasmatic cortisol, cortisol after low-dose dexamethasone-suppressing test and basal ACTH were respectively 851 nmol/l (287–2373), 529 nmol/l (169–1515 nmol/l), and 119 pg/ml (59–328). Pituitary lesion on MRI was a microadenoma (diameter < 1 cm) in 54.4% of cases ($n = 12$) and a macroadenoma in the other cases.
Results
A pre-existing diabetes was present in 8 patients with a mean HBA1C at 11.4% (10.8–12%). In the other patients, fasting plasma glucose and OGTT were performed and reveled five cases of normal glucose tolerance, four of pre-diabetes, and four of diabetes.
Comparative study between patients having normal glucose tolerance ($n = 5$) and those with pre-diabetes or diabetes ($n = 16$) showed that patients having glucose tolerance anomalies had a higher age (36.5 vs 26.8 years, $P = 0.047$) and more frequent catabolic signs (16/16 vs 2/5; $P = 0.008$) than patients without glucose anomalies. No other correlation was found between glucose tolerance and clinical, biological or radiological features.
Conclusion
Glucose tolerance anomalies are common in CD. These anomalies seem to be positively correlated to the age and to the presence of catabolic signs.

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P49

European Adrenal Insufficiency Registry: a comparative observational study of glucocorticoid replacement therapy

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Introduction
A once-daily modified-release formulation of hydrocortisone (Plenadren®) has been developed to better mimic physiological cortisol secretion in the treatment of patients with adrenal insufficiency (AI). Here, we describe EU-AIR, an ongoing post-authorization observational study (registry) designed to collect information on AI management and to assess the long-term safety of Plenadren® compared with traditional glucocorticoid replacement therapies in routine clinical practice.
Design
Patients with AI (primary or secondary), not participating in an interventional clinical study, are eligible for inclusion. The registry focuses on intercurrent illness, adrenal crisis, and serious adverse events. Other data collected include mortality, adverse drug reactions, dose changes, metabolic parameters, bone mineral density/content, and concomitant therapies. At enrolment, comprehensive demographic and baseline data, aetiology of AI and details of glucocorticoid replacement therapy, are collected electronically. Safety data and treatment information will be collected at subsequent clinic visits. The overall dataset will be analysed and, additionally, stratified according to patient subgroups (e.g. patients with hypertension, diabetes and/or other hormone deficiencies). All medical care decisions, including whether to treat with Plenadren® or conventional glucocorticoids, are made by the registry physician and patient.
Results
Approximately 20 centres in Germany, The Netherlands, Sweden, and the UK have been selected to participate in the study. Recruitment began in August 2012. As of 9 January 2013, the study had been initiated in eight centres and the total number of patients enrolled was 155. Primary analysis will be performed when 1800 patient-years of exposure have been achieved for each patient group (Plenadren® vs other replacement therapies). Reports will be provided to the European Medicines Agency every 6 months.
Conclusion
EU-AIR provides an opportunity to document evidence that will inform future clinical practice in the treatment of patients with AI.

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P50

Oncocytic adrenal carcinoma with production of testosterone and cortisol: case report

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We describe case of 29-year woman with no concomitant illneses in her history and with negative family history in sense of adrenal or cancer disease.
She came first in February 2012 for secondary hypomenorrhea lasting for 3 months, hirsutism and worsening of acne. Laboratory examination reveals markedly elevated free testosterone (9.6 nmol/l) and slightly elevated morning cortisol (687.4 nmol/l) with almost no suppresion in low dose dexamethasone suppresion test (1 mg) and elevated DHEA-S. On CT scan was diagnosed multinodular adrenal tumor 70×45 mm (60 HU) in right adrenal gland, left adrenal was normal. In lower part of right liver lobe was small hypodense locus 21×15 mm suspected to metastasis. Feochromocytoma was excluded by assessment of plazmatic metanephrines.
In April 2012 surgery was performed – wedge-shaped excision of liver tumor and right adrenalectomy, biopsy of liver was benign – cavernous haemangioma. Biopsy of adrenal gland was described in first reading as feochromocytoma. After sugery, menstruation cycle, hirsutism, acne, level of plasma cortisol, free testosterone and cortisol after suppresion were normalized. For clinical discrepance I initiated second reading of adrenal biopsy and the conclusion was oncocytic variant of adrenal carcinoma.
In June 2012 PET/CT scan was performed, with negative results. Synacten test after surgery showed sufficient cortisol reserve. Patient is now in our dispensarization, in conclusion with oncologist no adjuvant treatment was recommended.
In comparison with literature, testosterone-secreting oncocytic adrenocortical carcinoma was firstly described in 2010 in New Zealand and our case is probably the third case of this extremely unique malignancy in the world.

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P51

Prevalence of primary aldosteronism among hypertensive population in Trabzon City, Turkey

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Introduction
There have been no studies on the prevalence of primary aldosteronism (PA) in Turkey. In this study, the prevalence of PA among the hypertensive population was investigated.
Methods/Design
The study was conducted among hypertensive patients, aged 16–88 years, who visited the out-patient clinic of Endocrinology from January 10th, 2011 to September 30th, 2011. 768 of the 774 consecutive hypertensive patients volunteered to participate. The blood pressure, height and weight were measured in all patients. The duration of the hypertensive disease, antihypertensive drug therapy, other concurrent diseases and addictions (e.g. smoking) were determined in all patients. After an 8-h fast, blood samples were collected for the determination of blood urea nitrogen, serum creatinine, sodium, potassium, plasma aldosterone concentration (PAC), and plasma–renin activity (PRA). Care was taken to ensure normal potassium serum levels in all patients before serum aldosterone/PRA ratio (ARR) test and a liberal intake of salt.
Results
ARR was found to be positive (ARR >20) in 134 of the 768 patients. Saline infusion test (SIT) was used as the confirmatory test. PAC cut-off used in SIT was 10 ng/dl in this study. The confirmatory test was applied to all 134 patients with positive ARR. The confirmatory test was found to be positive in six patients (0.8%). The prevalence was determined to be 6/768 (0.8%). The prevalence was 4.6 and 1.7% upon PAC cut-off values of 5 and 7.5 ng/dl were used respectively. The median values for potasium were found to be 4.5 (3.1–6.4) and 3.5 (2.9–4.2) in essential hypertensive and PA groups respectively ($P<0.001$). Three of the six patients (50%) with PA had hypopotassemia.
Conclusion
The prevalence of PA was found to be 0.8%. More epidemiological studies are needed in the different regions of the country to evaluate the prevalence of PA in Turkey.

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P52

Adrenal metastases: aetiolgies and outcome

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Introduction
Adrenal metastases used to be rare in endocrinology compared to others adrenal tumours, and deemed to have a dire prognosis. Our aim is to analyze 13 cases in order to study their morphological aspects, their causes, and their outcome.
Subjects and method
Thirteen cases were studied over a long period of time (2000–2012). All of them had biological, hormonal, and radiological assessments.
Results
We had ten men and three women, mean = 58.2 (33–72). They consulted for an aching back or abdomen ($n=6$), and for weight loss ($n=3$). The adrenal mass was discovered incidentally by ultrasounds in 3 cases, and after an adrenal crisis in one case. On CT scan they were bilateral ($n=9$) and unilateral (right adrenal) in 4 cases. Mean size = 43.54 mm (11–100 mm). They were generally well limited (13), heterogeneous (3) and with low density (2), but much vascularised. For adrenal function, the cortisol was normal or very low, and sometimes high (error, stress, collision tumour??). Other metastases were located in bones ($n=3$), lymph nodes ($n=2$), brain (1), liver (1), pulmonary ($n=1$), and pituitary ($n=1$). They

survived 7.75 months (1–24).The primary cancer was: pulmonary ($n=8$), pleural ($n=1$), thyroid ($n=2$), and unknown origin (3).
Conclusion
Adrenal metastases are rare (1.08/year). They prevail in males. They are associated to other metastases, but they can be discovered incidentally. They are generally large, bilateral and much vascularized. For their cause, pulmonary cancer is in the first position, but all other cancers can be the cause. Their dire prognosis can be worsened by an adrenal crisis; for this they should be recognised very soon.

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P53

Adrenal inclusion in testicular: about six cases

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The adrenal enzyme deficiency leads pseudopuberty in later diagnosis or in the absence of treatment. The existence of testicular enlargement in boys can be related to adrenal inclusion. We report six observations about this pathology: Two brothers of 2 and 12 years old with 11β-hydroxylase and four patients of three, five, height and ten years old with 21-hydroxylase deficiency. The reson of consultation was the development of the penis and pubic hair with a testiculary enlargment.Hormonal balance was in favor of early pseudopuberty (FSH average, 0.15 µ/l; LH average, 0.02 µ/l; and testosterone average, 8 ng/ml). Testicular ultrasonography objectified increased volume and testicular hypoechogenes nodules. Tumor markers (βHCG and ACE) were negative. Replacement and suppressive therapy by glucocorticoids is indertaken. The evolution was marqed by regression of secondary sexual characteristics, reducted testicular size, increasing its echogenecity and loss of nodules. During a reevaluation ten years later, a large heterogeneous testicular nodule is found in the the fourth boy. Tumor maekers were elevated. Orchidopexy is decided.
The intratesticular adrenal inclusion is rare. It is the result of a cortical defect with a delay in diagnosis. Reduction of testicular volume after glucocorticoid therapy is a good predictor of disease control. However, a long term of follow up is necessary because a possibility of tumor degeneration is not exceptional.

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P54

Hyperaldosteronism in patients with hyperparathyroidism: three cases

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Introduction
Hyperaldosteronism can induce elevated parathyroid hormone (PTH) levels, presumably by increasing calciuria. Furthermore, PTH stimulates aldosterone secretion *in vitro*, and increases angiotensin–II-stimulated aldosterone release. In a patient with hyperaldosteronism and hyperparathyroidism, PTH receptors were detected in aldosteronoma tissue. We present three patients sent to an endocrinologist for treatment/follow-up of primary hyperparathyroidism, with resistant hypertension, in whom hyperaldosteronism was diagnosed. Aldosterone (ald) and renin (re) (RIA) are expressed in pg/ml. The captopril test (CAP) was performed per protocol: minimum 2 weeks on doxazosin as sole antihypertensive, minimum 133 mEq sodium intake for 3 days, basal ald/re (Bald/re), 1 and 2 h post-25 mg captopril. Test positive if 2 h ald >120 or ald/re >50.
Case 1
A 74-year-old male presented calcemia(Ca): 11.1 mg/dl, PTH (IRMA) 71 pg/ml. Office BP: 160/95 mmHg, on losartan (100 mg), amlodipine (10 mg), hydrochlorothiazide (25 mg), and atenolol (50 mg). Ultrasound: parathyroid adenoma. He rejects parathyroid surgery. Treatment: cinacalcet 30 mg/day. CAP: Bald/re $352/3=117.3$ 1 h:$339/2=169.5$, 2 h: $408/3=136$. He rejects adrenal catheterization/surgery. Treatment: eplerenone 50 mg b.i.d.
Case 2
A 68-year-old female is referred following parathyroidectomy for parathyroid hyperplasia. Pre-surgical Ca: 11.4 mg/dl, PTH(IRMA): 84 pg/ml, Office BP: 200/100 mmHg on losartan (50 mg), hydrochlorothiazide (12.5 mg), atenolol (50 mg). CAP: Bald/re $174/4=43.5$, 1 h:$224/4=55.5$ 2 h: $170/5=34$. The patient rejects catheterization/surgery. Treatment: eplerenone 50 mg b.i.d.
Case 3
A 81-year-old female presents Ca: 10.9 mg/dl, PTH (ChL) 129 pg/ml, Office BP: 175/90 mmHg on nebivolol (5 mg), lercardipine (20 mg), and furosemide (40 mg). Parathyroid disease not detected by Ultrasound. CAP: Bald/re $203/2=101.5$, 1 h: $129/6=21.5$, 2 h:$169/9=18.7$. Treatment: spironolactone (100 mg/24 h), cinacalcet (30 mg/day).
Conclusions
Three cases of hyperaldosteronism associated with hyperparathyroidism were detected in the clinic of a single endocrinologist over 2 years, suggesting that the association is not infrequent, and underlying the importance of ruling out hyperaldosteronism in patients with hyperparathyroidism and moderate/severe or resistant hypertension.

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P55

Analyses of data of the patients with Cushing's disease with use of Moscow Region's database

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Introduction
To analyze clinical, laboratory and anamnestic data of the patients with Cushing's disease (CD) we conducted a CD database.
Methods
One hundred and eighty patients with CD were investigated in MRRCI from 1975 year. To all of them in 2011 the invitation letter was sent. In only 22 patients we could confirm diagnosis of CD according to their medical documentation. Three patients died to 2011. From 2009 to 2012 in 16 patients CD were newly diagnosed. So, now in our database 38 pat. (35- female, 3 male) with laboratory and histologically confirmed CD.
Results
The age of patients at diagnosis were 39.2 ± 12.1 y.o. Duration of their complaints – 36 (22;75) mo. BMI – 34.2 ± 6.8, 71.5% of patients were obese. One patient noted weight decreasing. In 15.7% of cases relatives of the pat. had malignancies, in 13% – endocrine diseases. In 42% of patients additional endocrine diseases revealed. In 48% of pat. glucose intolerance was newly diagnosed (17% – glucose intolerance, 83% – DM). In all patients pathology of cardiovascular system were find (hypertension – 100% (sBP – 187 ± 30, dBP – 110 ± 16), heart failure – 11%, MI – 3%). Pulmonary embolism – 5%. Gastrointestinal diseases – 83%, urinary tract diseases – 24% (urolithiasis – 11%). Psychiatric disoders – 42.1%. Osteoporosis – 24%, osteopenia – 38%. Skin affection – 53% (mostly pityriasis versicolor). Infections of various organs – in 72% of patients. *Laboratory changes*. UFC was high in 66%, cortisol level after LDDST – $618,3\pm318,9$ nmol/l. Mean of cortisol suppression after HDDST – $80,9\pm18,1$%, suppression more then 50% revealed in 96% of pat. Pituitary MRI revealed macroadenoma in 14%, micro – 55%, no abnormalities – 31%. *Treatment.* The only neurosurgery (N) was done to 15 pat., radiotherapy only (R) – 5 pat., N + R – in 2 pat. Adrenalectomy + N/or R – in 14 pat. One pat. refused from surgical treatment and receive medication. *Efficacy*. Secondary adrenal insufficiency – 61%, remission – 13%. Relapse of CD – 23%. 1 pat. (3%) died before treatment.
Conclusion
The database shows high prevalence of different complications of CD, endocrine and non endocrine diseases in relatives, effectiveness of treatment. High prevalence of relapse after treatment tend to lifelong monitoring of the patients.

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P56

Evaluation of monitoring protocol for adrenal incidentalomas in our area

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Introduction
According to the latest recommendations, monitoring protocol for adrenal

incidentalomas includes: hormonal determinations annually for 4 years and imaging at 6, 12 and 24 months. The objectives of this study are: i) to define the clinical features, natural history and clinical management of adrenal incidentaloma in our area, ii) to evaluate the performance of the current monitoring protocol.
Methods
Retrospective study including patients diagnosed with adrenal incidentaloma between 2007 and 2012 in our area. Epidemiological and clinical data were analyzed, as well as the morphological and functional characteristics at diagnosis and during follow-up.
Results
Ninety six patients were included (55,2% male, mean age 61,38 ± 12,15 years). 74,3% were overweight or obese (mean BMI of 29,66 ± 4,94 Kg/m^2), 51% had hypertension, 32,3% dyslipidemia, 25% diabetes and 20,8% osteoporosis. 63,6% of the cases were detected by computed tomography, performed in most patients by digestive (21,1%) or genitourinary pathology (21%). 48,4% of adrenal incidentalomas were located in the left gland, 8,4% were bilateral and mean size at diagnosis was 26,6 ± 20,2 mm. 2 cases of Cushing's syndrome and two of pheochromocytoma were detected at the initial evaluation and six patients underwent surgery for larger than 40 mm incidentaloma. Biopsy confirmed a case of adrenal carcinoma. At two and four years of follow up, 98,86% maintained normal function (only one pheochromocytoma diagnosis at 6 months), and no significant changes in size were observed (mean resizing at two year evaluation was 0,56 ± 2,3 mm, $P = 0{,}103$).
Conclusions
In our area, no significant changes were detected in either the size or hormonal - function in incidentalomas initially diagnosed as non-functioning; so the efficiency of systematic application of current protocol should be reassessed.

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P57

Impact of antihypertensive drugs, sodium intake and potassium plasma concentrations on plasma aldosterone and plasma renin aktivity

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Introduction
Primary aldosteronism (PA) is a group of disorders which are characterized by inadequate and non-suppressible production of aldosterone. The prevalence of PA is increasing in hypertensive population. The golden standard of screening for primary aldosteronism, determination of aldosterone/plasma renin activity (ARR), is influenced by numerous exogenous and endogenous factors. Testing cannot always be conducted under optimal conditions.
Objective
To determine influence of antihypertensive drugs and concentrations of potassium and sodium in blood and urine on values of aldosterone and plasma renin activity.
Methods
In this retrospective study, we analyzed medical reports of patients admitted to Department of thyroid gland disease in the period from 2009 to 2011, with increased risk for primary aldosteronism. Body weight and height, sodium and potassium in serum and urine, plasma aldosterone concentrations and plasma renin activity, data on medicines and comorbidity were analyzed in all patients. In processing data, statistical methods descriptive analysis, Student *t*-test and univariate linear regression were applied.
Result
Of 137 patients, there were more patients with resistant hypertension (53,28%) than with adrenal tumors (46,72%). Most patients used calcium channel blockers. Treatment with alpha blockers and calcium channel blockers does not influence ARR. Beta blockers and ACE inhibitors can influence ARR and diuretics and vasodilatators have definite influence. Diabetes mellitus can have higher risk of false negative results. Urine sodium excretion is significantly correlated with plasma aldosteron and serum potassium. Plasma aldosteron and PRA are significantly correlated with concentrations of electrolites in urine.
Conclusion
Increased prevalence of primary aldosteronism necessitates need for accurate and better diagnostics.

Keywords: primary aldosteronism drugs sodium potassium correlations.
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P58

A case of Addison's disease caused by systemic disseminated tuberculosis: mimicking lymphoma on F-18 FDG PET-CT

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Addison's disease is most commonly caused by autoimmune adrenalitis. But, adrenal tuberculosis is still major cause in developing countries, that usually caused by hematogenous spread of pulmonary tuberculosis. Systemic tuberculosis causing Addison's disease has been rarely reported. We present a case of Addison's disease caused by systemic disseminated tuberculosis which was mimicked as lymphoma on F-18 FDG PET-CT (PET-CT).
Sixty eight-year-old woman visited with dyspnea for 3 days. She presented with about 10 kg of weight loss, general weakness, and nausea for 3 months. There were no history of pulmonary tuberculosis and medications like steroids. Her skin and oral mucosa were hyperpigmented and non-tender enlarged lymph nodes were palpated in both axilla and inguinal area. The levels of serum cortisol (< 1.0 μg/dl) and aldosterone (< 10 pg/ml) were low and the level of serum ACTH was high (843.50 pg/ml). Cortisol response was decreased in rapid ACTH stimulation test. Then, she was prescribed prednisolone 15 mg/day and fludrocortisone 0.05 mg/day. After few days of medication, her symptoms were started to improve. On fluid analysis of left pleural effusion, there were no evidences of tuberculous pleurisy or malignant cells. Multiple lesions with increased FDG uptake were seen in PET-CT: neck, intrathoracic, intraabdominal, axillary, iliac and inguinal lymph nodes, both palatine tonsils, adenoids, and both adrenal glands. In the view of these results, the lymphoma was highly suggested. The results of Core needle biopsy of left axillary lymph node was chronic granulomatous inflammation with negative PCR for *M. tuberculosis* or non-tuberculous mycobacterium. With positive result of Mantoux skin test, we clinically diagnosed as tuberculosis and started empirical antituberculous treatment. After 2 months of treatment, left pleural effusion was disappeared. After 9 months of treatment, the FDG uptaken lesions were disappeared in follow-up PET-CT. Now, she is free of symptoms with prednisolone 5 mg/day and fludrocortisone 0.05 mg/day.

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P59

Cardiovascular risk in Cushing's syndrome

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Introduction
Cushing's syndrome is rare but severe because of his association with multiple complications and particularly increased cardiovascular factors. This complications determine a mortality rate four times higher than in general population.
Objects
The aim of our study was to evalated the prevalence of cardiovascular factors in Cushing' syndrome.
Subjects and methods
Twenty-five patients with Cushing' syndrome were retrospectively evaluated from 2008 to 2011. All of them were evaluated for anthropometric (weight, height, waist circumference, biochemical profile (including fasting glucose and oral glucose tolerance test, lipids), and clinical blood pressure.
Results
The mean age was 35.6 years, and the sex ratio 4/1 (females/males).
86% of the patients were overweight and 57.3% had high waist circumference. 76.3% of the patients had hypertension, 69.5% had impaired glucose tolerance or diabetes, 48% had hypoHDLemia and 35.2% had hypertriglyceridemia.
Conclusion
Control of all cardiovascular risk factors should be one of the primary goals during the follow-up of these patients as remission from hypercortisolism is difficult to achieve and cardiovascular risk can persist even after remission.

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Management of adrenal carcinoma in a tertiary center of Endocrinology in Romania

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Adrenal cortical carcinoma is a rare disease with an incidence of 1–2/million per year. Diagnosis is related to autonomy of adrenal steroid synthesis, tumour size above 6 cm, local invasion and tendency to recurrence. Treatment consists in adrenal surgery, non-specific chemotherapy and radiotherapy, and, more specific-Mitotane (Lysodren).
Pathology diagnosis is based on capsular and vascular invasion, histological changes suggestive for malignancy and a high nuclear proliferation index.
We present a series of 7 cases diagnosed with adrenal carcinoma admitted in the last 3 years in the National Institute of Endocrinology, Bucharest.
The patients, 3 M and 4 F, aged 23–73 years, had large adrenal tumours 6–15.8 cm, locally invasive in liver, kidney, spleen, as proved by CT/MR imaging. The pre-surgical endocrine evaluation was available in 3/7, showing both glucocorticoid and androgen autonomous secretion. The rest were admitted after surgery for endocrine/oncological management. All cases were submitted to open surgery. Pathology data confirmed the clinical suspicion; Ki-67 immunostaining was available in 4/7 cases – between 15 and 25%. Two cases were submitted to chemotherapy and other 3 to radiotherapy, none to both. Mitotane became available in Romania in 2011, therefore 5/7 patients started this treatment, targeting the therapeutic levels 14–20 mg/dl. 4/5 reached therapeutic levels; 1 patient with extensive disease died before. Another patient died after surgery for local recurrence during Mitotane treatment, due to a fatal arrhythmia unrelated with the disease.
All the other are without any sign of morphological or biochemical recurrence after 7/11/19 month of Mitotane therapy. Glucocorticoid replacement therapy was associated in all cases; 2/3 developed primary hypothyroidism and L-T_4 substitution was started. Optimal management of adrenal carcinoma requires multidisciplinary approach and evidence of multicentric studies.

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Severe hyperglycemia due to cortisol producing adrenal carcinoma

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Introduction
Adrenal carcinomas are rare aggressive endocrine tumours with an incidence of 1–1.7 per 1 000 000. Sixty percent of patients present with symptoms of excess hormone secretion, most commonly as hypercortisolism, with or without virilisation due to accompanying androgen secretion. Tumours without clinical hormonal excess most often present with symptoms such as abdominal discomfort or back pain due to tumour growth.
Case report
A 72-year-old male patient with a history of type 2 diabetes mellitus was admitted to the hospital with severe hyperglycemia. Medical history revealed arterial hypertension and a lesion of the left adrenal gland (44×36 mm). Laboratory evaluation showed elevated levels for leucocytes, glucose and HbA1c as well as low levels of potassium. Initial chest X-ray showed a suspicious left hilus. Further studies, including CT thorax and MRI of the abdomen, showed evidence of multiple pulmonary, lymphatic, muscular and mediastinal metastases. Bone scintigraphy was inconspicuous. Owing to clearly progress of adrenal tumour size (62×46 mm), the patient underwent extensive endocrinological work-up. Blood tests showed elevated levels of cortisol, DHEA, androstendione and 17-OH-progesterone. ACTH was suppressed. Dexamethasone suppression test showed no suppression. In conclusion metastasised adrenal carcinoma was suspected. Biochemical evaluation was followed by a sonographically controlled biopsy of the mediastinal metastasis which confirmed the diagnosis of a hormononally active adrenal carcinoma. A chemotherapy with mitotane, 5-FU and streptozotocine was started, potassium was substituted. Hyperglycemia was controlled by an intensive insulin therapy. Hypercortisolism persisted but unfortunately the patient eventually decided against ketoconazole or any further therapy.
Conclusions
An adrenal carcinoma may present with severe hyperglycemia and hypokalaemia. Further investigations, including endocrine testing and imaging, are needed to detect the underlying tumour.

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Metastatic adrenocortical carcinoma presenting with concomitant secretion of glucocorticoid, mineralocorticoid, androgen, and catecholamines: a case report

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Introduction
We report the first case of metastatic adrenocortical carcinoma (ACC) concomitantly secreting cortisol, aldosterone, catecholamines and androgens with the extensive distant metastasis at the time of diagnosis.
Case report
A 57-year-old woman referred for left adrenal mass (5.5×4.0 cm) two years after the diagnosis of breast cancer. The patient's medical history revealed hypertension and diabetes mellitus. She has cushingoid and hyperandrogenemic appearance. Routine biochemical findings were normal, except for marked hypokalemia. Laboratory testing showed hyperaldosteronism, hyperandrogenism, hypercortisolism and increased urinary catecholamines; plasma renin: 170 pg/ml (2.71–16.51), plasma aldosteron: 678 pg/ml (29.4–161.5), basal ACTH: 7.8 pg/ml (0–46), cortisol: 27.2 μg/dl (5–25), DHEAS: 947 mg/dl (35–430), testosterone: 301 ng/dl (0–74), free testosterone: 8.23 pg/ml (0.29–3.18), androstenodion >8.6 ng/ml (0.3–3.7), 17 OH-progesterone: >20 ng/ml (0.08–1.3), urinary VMA: 16.6 mg/24 h (3.3–6.6) and urinary adrenalin: 43.8 μg/24 h (4–20). Loss of suppressibilility by both 2 and 8 mg dexamethasone and increased 24-h urinary free cortisol excretion (539 μg/24 h) suggested autonomous cortisol secretion. She uderwent left adrenalectomy and hepatic wedge resection for metastatic lession. Histopathologic diagnosis was reported as an adrenocortical carcinoma. Immunohistochemical examination showed positive staining of neoplastic cells for synaptophysin and inhibin, while tumor cells did not express S-100. Hepatic tumor histology was reported an ACC metastasis. Her laboratory results reached normal levels after operation. After the diagnosis of metastatic ACC, the patient received palliative chemotherapy, including carboplatin and etoposide. She was died 2 years after diagnosis.
Conclusion
ACC is a rare malignancy with a poor prognosis and presents with variable clinical pictures. Hormonally active adrenocortical carcinomas most commonly secrete cortisol while the co-secretion of multiple steroid hormones is rare. This is the first case of metastatis ACC in a patient with concurrent Cushing's syndrome, Conn's syndrome, hyperandrogenism and pheochromocytoma at the time of initial presentation.

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Adrenal lymphoma: about two cases

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Lymphoma adrenal is a rare cause of adrenal tumor (0.5%). Bilateral primary lymphoma adrenal phenotype T is exceptional. We report two observations. MO 56 years old was hospitalized FOR exploration and therapeutic management of two large adrenal masses discovered on CT imaging after back pain, and weight loss. Physical examination revealed a patient asthenic, with no signs of hypersecretion. The rest of the examination was unremarkable and research call signs primary neoplasm was negative. A hormonal balance showed a low cortisol with a height ACTH level. This which required start hormone replacement therapy to hydrocortisone. The MRI objectivied large masses without signs of infiltration and locoregional lymph nodes without root. The patient was operated. However, given the highly invasive mass that extended, biopsy is performed.
GA 31 years old has an adrenal mass revealed on the occasion of pain abdominals. She had a personal history of non Hodgkinphenotype B lymphoma treated during childhood. The clinical examination was without abnormalities. The MRI showed a heterogenous mass of 46 mm and multiple nodes. The patient receives chemotherapy. A reduction of tumor volume and disappearance of lymph nodes were observed. Additional surgery is performed. The histological study was in favor of NHL A predominance of diffuse large B cells is observed. The type T is exceptional. The clinical signs are not specific. Frequently, adrenal insufficiency

is observed indicating almost complete invasion of the adrenal gland. It is often asymptomatic which justifies its systematic research.

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Hypercortisolism endogenous and pregnancy: a report of three cases

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The occurrence of Cushing' syndrome in pregnancy is a rare entity due to hypogonadism. Its diagnosis and treatment are very difficult because pregnancy causes hormonal and physical changes which can lead to confusion. In addition, the therapeutic opties are limited because of reduced fetal risk. We rereport the observations of three patients who presented severe Cushing' syndrome appeared in the first part of pregnancy. This is unkown despite a very evocative painting. Hypercorticism is complicated by diabetes mellitus, arterial hypertension and death fetal *in utero*. The paraclinical exploration was in favor for no ACTH dependant hypercortisolism in two cases with a voluminous adrenocortical carcinoma (15 and 20 cm) and metastatic liver and bone and adrenal adenoma (6 mm) in the third patient. Adrenalectomy was realized to her while partial tumor excision was practiced in the others. Complementary therapy (anticortisolic mitotic and chemotherapy) is realized. The diagnosis of Cushing's syndrome in a pregnant woman must be recognized early cause of morbidity and mortality maternofetal increased. If signs can be attributed to pregnancy such weight gain, hypertension or diabetes mellitus, other elements should attract attention as signs of hyperadrogenism, or signs of hypercatabolism. The adrenal adenomas and adrenocortical carcinomas are the two main cases. The reason for this preponderance is unclear: less hypogonadism?, HCG?, oestrogens?, progesterone?. If adenoma is effectively treated by surgery, pregnancy is very harmful scalability and prognosis of adrenocortical whose resection is often difficult and incomplete.

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Adrenal Medulla

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Stud phenotypic study of paraganglioma extra surrenlien: about height cases

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Paraganglioma is a tumor developed in expenses of the sympathetic or parasympathetic nervous system. It is a rare pathology (prevalency 1 for 30 000). These are situated on the basis of skull, at the level of neck, in the thorax and the belly. Some paraganglioma are functional and secrete cathecolamines and others are diagnosed after surgery in the histological study of the operating piece. Through a retrospective study concerning height patients having paraganglioma extra surrenalien brought together in 20 years. We have specified theirs phenotypes.

Results

Reason of consultation: HTA (60%), abdominal pain (40%).
Mean age: 46.5 years (17–82), sex ratio: 1,6.
Clinical: HTA 12.5%. HTA, adrenergic signs (37.5%) signs tumors: 25%.
Hormonal balance: non functional: 37.5%; functional: 62.5%.
Imaging localization: Cervical: 1; Mediastinal: 1; Retroperitoneum: 5; Multiples: 1.
Size tumor: large ($\geq$4 cm): 62.5%.
Surgery: Recurrence: 25%.
Neoplasia 1 case
Genetics 1 case

The paraganglioma is a tumor with various phenotypes. Theses are voluminous or smalls, uniques or multiple, secretant or no and have a risk of recurrence and degenerescence. This fact, it may be requiring a long term surveillance. Because of the family character of this pathology (1/3 cases) a genetic study is proposed at every patient in particulary if he young, has multiple localizations or presents a cunning shape.
Motif de consultation: hta severe (60%) douleurs abdominales (40%)
Age moyen: 46.5ans (17–82) sex e 3F 5 H sex ratio F/H: 1.6
Clinique: hta (12.5%)
htaet signes adrenergique (37.5%)
signes tumoraux: (25%)
bilan hormonal non fonctionnel 37.5%
Bilan fonctionnel 62.5%
IMAGERIE: localisation cervicale: 1 cas (12.5%)
Localisation médiastinale: 1 cas (12.5%)
Rétropéritoine: 5 (62.5%)
Localisation multiples 1 (12.5%) et de petites tailles
Taille tumorale volumineuse ($\geq$4 cm): 62.5%
Chirurgie: Récidive: 25%
Néoplasie: UN cas
Génétique: UN seul cas

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Plasma free metanephrine and normetanephrine in chronic kidney disease patients

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Chronic kidney disease (CKD), with or without dialysis, often prompt biochemical tests for pheochromocytoma diagnosis due to high blood pressure, hemodynamic instability or association with certain genetic syndromes. Our objective was to assess plasma free metanephrine and normetanephrine in CKD disease patients, with or without dialysis.

Methods

Plasma free metanephrine and normetanephrine were measured by an enzyme-linked immunosorbent assay in 42 patients with CKD (23 on hemodialysis, six on peritoneal dialysis and 13 with stage 3–5 CKD but without dialysis), 30 patients with histologicaly proven pheochromocytoma and 43 control hypertensive patients. An adrenal mass was ruled out by an abdominal CT scan in all CKD and control hypertensive patients. As the upper limit of normal for both metanephrine and normetanephrine we used the manufacturer provided values. Values are presented as median (25, 75 percentile). Mann Whitney *U* test was used for group comparisons. For multiple testing Bonferroni correction was used.

Results

Both free metanephrine and normetanephrine levels were significantly higher in hemodialysis (90 (52, 162) and 303 (154, 356) pg/ml), peritoneal dialysis (69 (32, 100) and 312 (1940, 370) pg/ml) and CKD patients without dialysis (41 (22, 52) and 129 (68, 171) pg/ml) than in control hypertensive group (18 (13, 26) and 54 (25, 74) pg/ml) and significantly lower than in pheochromocytoma group (247 (91, 548) and 1329 (698, 1972) pg/ml). Dialysis patients (hemodialysis plus peritoneal dialysis) have higher plasma metanehprine and normetanephrine than CKD patients without dialysis. 48 and 65% of hemodialysis patients had plasma values of metanephrine and normetanephrine respectively over the manufacturer provided upper limit of normal.

Conclusion

Plasma free metanephrine and normetanephrine levels are frequently elevated in CKD patients, particularly in those on dialysis. Plasma free metanephrine levels rise well in the pheochromocytoma range.

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Succinate dehydrogenase subunit B mutations modify human neuroblastoma cell metabolism and proliferation

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Paragangliomas (PGLs) are rare neuroendocrine tumors. About 30–40% of these tumors are mutated in different susceptibility genes, including those

encoding the different subunits of the succinate dehydrogenase, a complex involved both in the tricarboxylic acid cycle and in the oxygen transport chain. The aim of this project was to investigate whether SDHB mutations may account for alterations in cell metabolism and functions. Since PGL cell lines are not available, we used the neuroblastoma cell line (SK-N-AS) stably transfected with the wild-type human SDHB, or different SDHB mutated constructs carrying the most significant mutations found in our patients affected by PGLs.
Interestingly, we found that all the SDHB mutated cell clones showed a specific reduction of the SDH enzyme activity. They also showed reduced oxygen consumption. Surprisingly and unexpectedly, in all the SDHB mutated clones we found a significant decrease in glucose uptake and in lactate concentration in the culture medium, while ATP production, was significantly higher, thus suggesting a shift in the utilization of the lactate metabolite towards glucose for energy production. Finally, we found that these metabolic changes are associated to increased potential in cell proliferation and migration. Overall, these data demonstrate that SDHB mutations deeply affect cellular metabolism and functions.

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Adrenal ganglioneuroma in a patient with a non-Hodgkin lymphoma

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Adrenal ganglioneuroma (AGN) is a rare neoplasm derived from the neural crest. It can be found along the paravertebral sympathetic plexus. Mean age at presentation is below 20 years. Most patients with AGN are asymptomatic and diagnosis is usually incidental.
Case report
Fourty three-year-old man with a personal history of hypertrophic cardiomyopathy in whom a follicular small cell non-Hodgkin lymphoma (NHL) was diagnosed after a biopsy of a left supraclavicular node. Initial extension study was conducted in December 2010 that included: a whole body CT, which showed multiple infiltrative lymphadenopathy and MRI of abdomen, where a 3.5×3 cm, well defined, spherical and homogeneously hypodense right adrenal mass was found. Also, PET scan confirmed multiple infiltrative lymphadenopathy, as well as infiltrative subcutaneous implants on both sides of the neck. Hormonal study was performed to exclude hyperfunction of the adrenal mass, which included: 24-h urinary free cortisol, 1-mg overnight dexamethasone suppression test, morning plasma cortisol, ACTH, renin, aldosterone, plasmatic catecholamines and its metabolites in plasma and urine, all of them within the normal range. After excluding hypefunction of the adrenal mass and not being able to exclude secondary infiltration of NHL, chemotherapy for treating NHL was started. After completing chemotherapy cycles no changes in size or morphology were seen in the adrenal mass, suggesting that it was independent of NHL. Consecutively, a laparoscopic right adrenalectomy was performed. Pathological study showed an AGN.
Conclusions
This is the first case of AGN described in a patient with a NHL. Adrenal masses are common findings in patients with hematological diseases that may be secondary to metastasic infiltration in up to 25% of cases. However, it should take into account all possible causes of adrenal masses, including the AGN.

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Evaluation of patients with pheochromocytoma for 10 yearly period

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Background and aims
Pheochromocytomas arise from chromaffin cells of adrenal medulla and sympathetic ganglia. It occurs in about 0.05 to 0.1% of patients with sustained hypertension. It is considered that about half the patients with pheochromocytoma have only paroxysmal hypertension. Pheochromocytoma is usually suggested by the history in a symptomatic patient, discovery of an incidental adrenal mass, or the family history in a patient with familial disease. We retrospectively analyzed all patients with pheochromocytoma for last 10 years.
Cases
Patients with pheochromcytoma (*n*: 35) who had data available for complete analysis were enrolled. All patients were evaluated in terms of complaints, age, sex, tumor localization, tumor origin, tumor size, recurrence and presence of MEN syndrome.
Results
Twenty-six patients (74.3%) had adrenal incidentaloma and remaining nine patients (25.7%) were symptomatic hypertensive. The average age at diagnosis was 48.54 (±15.47) years. Twenty-two (62.9%) patients were female and 13 patients were male (37.1%). Two patients (5.7%) had paraganglioma, remaining 33 patients had adrenal localization (94.3%). We diagnosed composite tumor (pheochromocytoma and ganglioneuroma) in two patients. In the majority of patients tumor was right adrenal localization (18 patients, 54.5%). On the other hand, 12 patients (36.6%) had left adrenal localization and three patients (9%) had bilateral pheochromocytoma. Medullary thyroid cancer (MEN 2) were determined in five patients (14.2%). Of five patients with MEN syndrome four patients were female. Mean tumor size was 6.36±3.11 cm. We determined recurrence in 5 female, 1 male, total six patients (17%). From the patients with recurrent, 2 female patients had also MEN two syndrome.
Conclusions
Our patients had female predominance. Majority of patients were admitted with adrenal incidentaloma. Increased use of radiographic imaging may be responsible for this result. Lots of adrenal masses were located in right adrenal gland. And average tumor size was larger than previously reported cases.

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Bone and Osteoporosis

P70

Bone loss in inflammation-mediated osteoporosis: a role for the P2×7 receptor?

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Bone loss in chronic autoimmune inflammatory diseases is a major clinical problem. Inflammation-mediated osteoposis (IMO) is the first animal model of generalized osteoporosis resulting from inflammation. The P2×7 receptor, an ATP-gated ion-channel, is primarily expressed on immune and bone cells. ATP is now seen as a novel inflammatory mediator, with P2×7 as main target of the pro-inflammatory activity. The P2×7-receptor has a regulatory role in bone formation and – resorption. In addition, P2×7-receptor knock-out (KO) mice have shown an attenuated immune response.
Aim
To investigate the role of the P2×7 receptor in IMO.
Method
Fourteen-week-old male mice entered the IMO protocol (75 WT and 75 KO). Fifteen mice were sacrificed at baseline, 30 mice injected with silica (Talc) were sacrificed at 10 and 20 days, (15 mice at each time point). The remaining 30 mice were injected with vehicle and sacrificed at 10 and 20 days, (15 mice at each time point). The experiment was repeated with P2×7 KO mice (Pfizer). Bone mineral density (BMD) was measured by PIXImus. Spleen was collected and weighted. Blood, spine and hind legs were collected for further analysis; strength test and histomorphometry. Data: mean±S.D.
Results
In WT animals, the spleen weight was higher in the Talc injected mice (WT: baseline 86±13 mg, vehicle 109±32 mg and Talc injected 131±44 mg). No difference in KO.
Spine BMD was significantly lower at 20 days compared to baseline in WT vs Talc (ANCOVA weight corrected: $P=0.032$). Moreover, at 20 days spine BMD was significantly lower in the Talc WT group compared to vehicle (0.046 vs 0.051 g/cm: $P=0.009$). In the KO animals no significant difference was found at 20 days between vehicle and Talc group (0.052 vs 0.052 g/cm) (ns).
Conclusion
P2×7 might be involved in the inflammation-mediated osteoporosis.

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Abstract withdrawn.

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Circadian rhythm of circulating sclerostin in healthy young men

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Introduction
It is recognised that osteocytes, by secreting sclerostin plays a more central role in bone homeostasis. Sclerostin is a physiological inhibitor of bone formation. By binding to the LRP5/6 receptor sclerostin inhibits the Wnt signalling pathway. A cross-sectional study was undertaken to ascertain whether an endogenous sclerostin circadian rhythm exists in healthy individuals.
Subjects and methods
Six healthy young men with normal BMD were admitted overnight. Blood samples were drawn every hour for 24 h samples obtained centrifuged immediately. The serum/plasma was separated and frozen at −70 °C for later analysis. An enzyme linked immunoassay (Biomedica, Austria) was used to measure sclerostin. All samples were assayed in duplicates. The inter and intra assay CV% being 12.3 and <5% respectively.
Statistical analysis
CHRONOLAB 3.0 (Universdade de Vigo, Vigo, Spain) a software package validated for analyzing biological time series by least squares estimation was used to analyze individual and population mean cosinor. The following circadian rhythm parameters were evaluated: 1) midline estimate statistic of rhythm (MESOR), 2) amplitude and 3) acrophase. A p value for the rejection of the zero-amplitude (no rhythm) assumption is determined for each individual and for the group.
Results
A definite sclerostin circadian rhythm was identified in the study population ($P=0.028$). The mean sclerostin (MESOR) was 84.4 ± 1.4 pmol/l. A nocturnal sclerostin peak (time of onset 0100 h) with concentrations remaining above mean over 4 h was observed. A small but ill sustained increase is noted at 0700 h which culminates in a steep descent to a trough (at 0800 h) the levels remain low throughout the morning till midday (1200 h). The maximum percentage increase in the sclerostin concentration between 0100 h and 0700 h ((value at each time point – 0100 h value)/ 0100 h value × 100)) was 19.7%.
Conclusion
Our results demonstrate a circadian rhythm with a nocturnal peak in the sclerostin secretion for young healthy men.

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Cross-correlation of circulating sclerostin over 24 h to PTH, phosphate and bone markers in healthy young men

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Introduction
Osteotropic hormones demonstrate circadian rhythms which are integral to bone homeostasis. Sclerostin is a physiological inhibitor of bone formation. We have established that Sclerostin has a distinct circadian rhythm with a nocturnal peak. Analysis was performed to determine the relationship of Sclerostin levels to PTH, Calcium, Phosphate, βCTX and P1NP in healthy young men.
Methods
Six healthy young men with normal BMD were admitted to our research facility. Blood samples drawn hourly for 24 h from 1400 h were centrifuged immediately. The serum/plasma separated was frozen at −70 °C. PTH, Calcium, Phosphate, βCTX and P1NP were measured. An enzyme linked immunoassay (Biomedica, Austria) was used to measure Sclerostin.
Statistical Analysis
Cross-correlational analysis was performed to determine the relationships between the 24 h profiles for Sclerostin, PTH, Calcium, Phosphate, βCTX and P1NP. This determines the correlation between two time series of equal length that have been paired, data point by data point, then one of the time series is shifted by one or more time points (lag time) and the correlation process is repeated. Time series for the groups were derived by calculating the mean value at each time point for all subjects.
Results
Secretory patterns of sclerostin Vs. PTH and Calcium demonstrate no definite correlation during the 24 h period. A positive correlation was noted between sclerostin and phosphate, βCTX, P1NP with correlation co-efficients of 0.637, 0.627, 0.666 respectively. The changes in the sclerostin preceded βCTX by 1 h, but zero lags between sclerostin Vs phosphate and P1NP.
Conclusion
Our results indicate, despite the existence of a circadian rhythm for sclerostin it does not seem to either directly influence or be influenced by PTH secretion. However the strong correlation of sclerostin to bone markers and phosphate with a zero lag indicates a direct influence of yet another factor on sclerostin circadian rhythm and bone homeostasis.

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Bone quality, as measured by trabecular bone score (TBS), in patients with primary hyperparathyroidism

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The fracture risk in primary hyperparathyroidism (PHPT) is partially independent of bone mineral density (BMD) and seems to depend on decreased bone quality, which is still reliably assessed only with invasive techniques. Trabecular bone score (TBS) is a grey-level texture measurement acquired during a dual X-ray absorptiometry (DXA) lumbar spine scan and it has been recently proposed as index of bone quality. This study is aimed to assess the role of TBS in predicting vertebral fractures (VFx) in PHPT patients.
We prospectively enrolled 92 PHPT patients (18 eugonadal males and 74 post-menopausal females, aged 62.7 ± 10.1 years) and 98 age–, gender– and BMI matched controls. In all subjects, TBS and BMD at spine (LS) and femur (FN) were assessed (reported as Z-scores) by DXA and VFx by X-ray. Among the 92 PHPT patients, we also report the available longitudinal BMD, TBS and VFx data after 24 months of follow-up for 20 subjects operated on and for 10 conservatively treated.
PHPT patients had lower BMD (LS -0.74 ± 1.14, FN -0.67 ± 0.84) and TBS (-2.39 ± 1.8), and higher prevalence of VFx (43.5%) than controls (0.51 ± 1.46, 0.05 ± 0.85, -0.98 ± 1.07 and 8.2%, respectively, $P<0.0001$). The presence of VFx was associated with TBS (OR 1.6; 95%CI 1.2 – 21, $P<0.001$) regardless of LS BMD, age, BMI and gender. TBS showed the best compromise between sensitivity (75%) and specificity (61.5%) for detecting VFx as compared to BMD (LS 31% and 75%, FN 64% and 65%, respectively). After 24 months, in the 20 patients surgically treated, no new VFx occurred and BMD tended to increase (Z-score change LS 29.9 ± 34.1% and FN 30.2 ± 39.3%, P=NS), while TBS increased significantly (52.8 ± 46.6%, $P=0.004$). In the 10 patients conservatively treated BMD and TBS tended to decrease (Z-score change LS -11.1 ± 46.1%, FN -29.7 ± 85.3%, TBS -139 ± 235%, P=NS). In the 3 patients with a new VFx, TBS decreased significantly (Z-score change -441.6 ± 217.9%) as compared to those without new VFx (-9.4 ± 14.9%, $P<0.05$), at variance with BMD (LS -40 ± 10% vs 1.4 ± 50% and FN -36.7 ± 103% vs -13.3 ± 16.1, P=NS).
Conclusions
In PHPT, bone quality, as measured by TBS, is reduced and improves after surgery. TBS appears to be more useful than BMD in detecting PHPT patients at risk of fractures.

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P75

Calcium homeostasis in women with non-metastatic breast cancer with osteoporosis after a single-dose of denosumab: a pilot study

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Introduction
Women with non-metastatic breast cancer form a distinct subpopulation in which calcium homeostasis in response to treatment with denosumab has not been extensively investigated.
Methods
Female patients with osteoporosis, who were eligible for treatment with denosumab, were prospectively enrolled (2011–2012) and divided into two groups; Group A consisting of patients with either no history or benign diseases non affecting bone metabolism ($n=24$ controls) and Group B patients with non-metastatic breast cancer ($n=18$). Documentation of renal impairment, disorders of parathyroid function or the presence of bone metastases served as exclusion criteria. All patients were administered a single-dose of denosumab under standard calcium and vitamin D supplementation. Serum calcium, phosphorus, parathyroid hormone (iPTH) and 24 h urine calcium were measured at days 0, 7 and 180. Primary outcomes were the development of hypocalcaemia and secondary hyperparathyroidism.
Results
At baseline, groups were comparable in age, calcium and iPTH levels. No events of hypocalcaemia were recorded. Overall, incidence of secondary hyperparathyroidism was found to be 45.5% one week after administration of denosumab. Interestingly, at day 180 incidence of secondary hyperparathyroidism was higher in Group B in contrast to the pattern recorded in controls, although not reaching statistical significance. At day 7, iPTH was found to be significantly higher only in controls (Wilcoxon Signed Rank test: $P=0.013$) compared to group-specific baseline values. At day 180, borderline increase in iPTH of Group B was noted ($P=0.08$), whereas iPTH returned to baseline in controls.
Discussion
A pattern of delayed development of secondary hyperparathyroidism might be present in patients with non-metastatic breast cancer. It could be extrapolated that this finding might be associated with a partial functional defect in calcium sensing receptor, which has recently been implicated in the pathogenesis of breast cancer. The findings warrant further investigation.

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P76

Long-term prospective evaluation of the effects of estroprogestagen therapy on the bone mineral density of girls with Turner syndrome carrying various *PvuII* AND *XbaI* polymorphisms of ER-α

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Introduction
Reduced bone mineral density (BMD) is present in many women with Turner syndrome (TS), while hypo-estrogenism is known to play a vital role in bone mineralization disturbances. It has been suggested that genetic factors play an important role in the regulation of BMD. The aim of this study was to analyze the association between PvuII and XbaI ER-a polymorphisms and BMD in TS patients subjected to estroprogestagen (EP) treatment.
Material and methods
Fifty-four TS patients aged 17–38 (mean age 22.7 ± 8.2), along with 82 healthy controls were the subjects for this study. Baseline values of hormonal parameters, BMD and bone density markers were measured in the subjects. Subsequently, TS patients underwent 5 years EP therapy.
Results
The results of laboratory parameters and BMD were analyzed in regards to *PvuII* and *XbaI* polymorphic variants of the ER-α gene. The increase in BMD of TS subjects was the highest in the first (7.5%, $P=0.013$) and second (6.6%, $P=0.008$) years of treatment. Four years of EP therapy was reflected by a significant increase in BMD *z*-scores in patients with *xx* and *Xx* genotypes of the *XbaI* gene and in those with with the *pp* and *Pp* genotypes of *PvuII*. In patients with haplotypes other than *XXPP*, BMD *z*-scores were significantly higher compared to their baseline after 2 ($P=0.002$), 3 ($P=0.001$), 4 ($P=0.002$) and 5 ($P=0.001$) years of treatment.
Conclusions
Genotypes *xx* and *pp* were shown to be prognostic markers of a good response to EP treatment, while the *XXPP* haplotype carriers were revealed to have the risk factors for insufficient responsiveness against EP treatment in BMD control.

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P77

Cathepsin K secretion in human primary osteoblastic cell cultures and its influence by different glucose concentrations

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One of the causes of secondary osteoporosis is type 1 DM (1DM), an autoimmune disease where insulin replacement is mandatory for controlling blood sugar levels. Among the mechanisms involved in osteoporosis in 1DM is increased bone resorption, mainly osteoclasts being responsible for this process. However, osteoblasts also seem to have an active contribution by secreting products with resorptive properties, one of these being cysteine protease Cathepsin K, as mentioned by a few studies.
Aim
Our study was directed towards assessing the secretion of Cathepsin K in human primary osteoblastic cell cultures and observing how different glucose concentrations in culture media influence the levels of this enzyme.
Material and methods
Primary human osteoblastic cell cultures were obtained using bone from patients with total hip replacement interventions. After reaching subconfluence in the third passage, cells were treated with several glucose concentrations: 2.8 mmol/l (hypoglycemia), 5.6 mmol/l (normoglycemia), 11.1 mmol/l (moderate hyperglycemia) and 28 mmol/l (extreme hyperglycemia). After 24 h of incubation in these particular media, supernatants were collected and Cathepsin K was measured quantitatively (ELISA).
Results
Cathepsin K levels obtained were: 31.04 ± 0.73SD pmol/l (moderate hyperglycemia), 31.54 ± 2.8SD pmol/l (extreme hyperglycemia), 34.78 ± 7.56SD pmol/l (normoglycemia) and 35.51 ± 6.97SD pmol/l (hypoglycemia). A trend towards lower Cathepsin K levels in both hyperglycemic supernatants compared to hypoglycemic and normoglycemic conditions was observed, however, these differences were not statistically significant (hypoglycemia–moderate hyperglycemia: $P=0.74$; hypoglycemia–extreme hyperglycemia: $P=0.8$; normoglycemia–moderate hyperglycemia: $P=0.83$; normoglycemia–extreme hyperglycemia: $P=0.88$).
Conclusion
Our experiment proves the secretion of Cathepsin K by osteoblasts; it can be speculated that the lower levels in both hyperglycemic supernatants may be explained by the growth-inhibitory effect of high glucose on osteoblasts but further studies on the topic are warranted.

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P78

Effects of Denosumab treatment on insulin resistance in postmenopausal women with osteoporosis

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Introduction
Denosumab is a new pharmacotherapy option for postmenopausal osteoporosis. It is a human monoclonal antibody against RANKL, acting as an osteoprotegerin (OPG) analog. Recently, osteoprotegerin levels were found to be elevated in type

two diabetes while insulin resistance was shown to be positively associated with decreased serum OPG levels, in healthy obese subjects. However, no study has been conducted in order to clarify the effect of anti-RANKL factors administration on insulin resistance, especially in women with postmenopausal osteoporosis.
Methods
The study population consisted of 20 non obese, non diabetic, postmenopausal women with BMI: 22.15±2.16 and age: 64.7±7.96 years. All patients had postmenopausal osteoporosis and were followed in our outpatient clinic for at least 18 months. Serum samples were collected and analyzed for fasting glucose, insulin and c-peptide levels before the initial dose of denosumab and at 6 and 12 months during treatment. HOMA index for insulin resistance (HOMA-IR) and steady pancreatic β cell function (HOMA-β) were calculated. Patients with 25(OH)D deficiency were excluded from the study. Statistical analysis was performed by using SPSS 15.0 software. Comparisons between groups were made by using ANOVA and unpaired *t*-test.
Results
During the study, patients' weight and 25(OH)D levels remained unchanged ($P=0.749$ and $P=0.456$ respectively). Denosumab treatment did not cause any statistically significant change on HOMA-IR ($P=0.194$) or HOMA-β values ($P=0.507$), at all timepoints of the study.
Conclusions
These preliminary data show that in non obese and non diabetic postmenopausal osteoporotic women, treatment with denosumab does not seem to have an effect on insulin resistance or steady pancreatic β cell function.

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P79

Impact of the FRAX® tool and the NOGG guidelines on the indication of bone mineral density in Spanish postmenopausal women

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Introduction
The National Osteoporosis Guideline Group (NOGG) recommends determine BMD in patients who present an intermediate risk of osteoporotic fracture using the FRAX® tool, follow-up for low risk, and treatment for high risk. In 2011, 3163 BMD were performed in the Complejo Asistencial Universitario de León (CAULE). Since the cost for each BMD is 58.6* (BOCYL 31/01/2011), annual spending could rise to *185 352. The aim was to assess whether fracture risk calculation would result in a reduction of BMD measurements made, and therefore in the costs.
Methods
Cross-sectional study in patients referred for BMD measurement in CAULE between April and December 2012. Data of risk factors included in the FRAX® were obtained using a questionnaire completed by the technical staff. The absolute risk of presenting a major or a hip fracture was calculated using the British FRAX® formula and NOGG guidelines. We excluded patients with current or previous treatments for osteoporosis.
Results
We recruited 1163 patients, of whom 542 (46.6%) were untreated. 95% were women, with a median age of 60.2 years (Interquartile Range, IQR=14.44). 20.1% had previous clinical fractures, 17.2% were smokers, 15.3% had parents with hip fractures, 8.3% had rheumatoid arthritis, 15.7% referred risk factors for secondary osteoporosis, 9.4% were on corticosteroids and 2.2% had a high-risk alcohol consumption. The median risk for major fracture was 8.2% (IQR=9), and for hip fracture 1.3% (IQR=3). Applying the FRAX tool in combination with the NOGG guidelines 55.5% had low fracture risk, 34.5% intermediate risk and 10% high risk. Extrapolation of the data shows that 30% of all BMD annually performed in CAULE could be avoided. This would mean a cost reduction of *55 605/year.
Conclusion
The application of the NOGG guidelines led to a decrease in BMD indications, reducing costs and improving the efficiency in management of osteoporosis.

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P80

Increased IL17A serum levels associated with low estrogen levels can play a role in postmenopausal osteoporosis

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Postmenopausal osteoporosis is characterized by lower bone mass, loss of structural integrity, and sometimes becomes life-threatening. The development of postmeno-pausal osteoporosis is multifactorial. Estrogen plays a preventing role in the bone resorption inhibiting the secretion of proinflammatory IL1 and TNFα cytokines. IL17 proinflammatory citokine is a new candidate in the pathogenesis of osteoporosis. IL17 levels are increased in estrogen deficiency.
The relationship between serum IL17A levels and estradiol levels, as well as bone mineral density (BMD) was studied in 72 post- and 22 premenopausal women. Enzyme-linked immunosorbent assay for IL17A and chemiluminescence method for estradiol detections were used. Dual energy X-ray absorptiometry (DXA) was applied for BMD measurement in the lumbar spine (L1–L4) and femoral (total and neck) regions.
The estradiol levels were significantly higher in premenopausal women compared with postmenopausal ones (239.44±226.17 vs 74.21±4.44 pmol/l, $P<0.0001$). Increased IL17A levels were demonstrated in postmenopause in comparison with those in premenopause (3.5±0.56 vs 2.88±0.08 ng/ml, $P<0.0001$). Seventy eight women out of 94 had lower estradiol levels (<80 pmol/l) and demonstrated elevated IL17A levels in comparison with 16 women who had estrogen levels in the normal range (3.43±0.56 vs 3.01±0.38 ng/ml, $P<0.0001$). IL17A levels were higher in osteoporotic women than in osteopenic ones (3.65±0.61 vs 3.31±0.0.08 ng/ml, $P<0.013$ in lumbar region, and 4.19±1 vs 3.46±0.51 ng/ml, $P<0.015$ in femoral region). In postmenopause, the differences in BMDs between women with low and high (>3.04 ng/ml=mean of all women+2S.D.) IL17A levels were significant in femoral region, particularly in femoral neck region (0.72±0.1 vs 0.64±0.14 g/cm^2, $P<0.007$). A strong correlation was demonstrated between IL17A levels and BMDs of all studied regions in postmenopause.
The results highlighted a relationship between IL17A and estradiol levels, as well as BMDs. Estrogen deficiency in postmenopause can accelerate the bone-wasting through increased IL17A levels, particularly in femoral neck region.

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P81

Effects of the tamoxifen on calcitonin producing thyroid C cells and bone in rat model of male osteoporosis

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Thyroid C cells produce bioregulatory peptid calcitonin (CT), which lowers plasma calcium (Ca) and acts as an inhibitor of bone resorption. In this study we investigated the effects of tamoxifen, as a selective estrogen receptor modulator on thyroid C cells and skeletal changes in middle-aged orchidectomized (Orx) rats as an animal model of male osteoporosis. Fifteen-month-old male Wistar rats were divided into Orx and a sham-operated (SO) groups. Two weeks after gonadectomy, one Orx group was injected subcutaneously (s.c.) with tamoxifen citrate (Orx+TAM; 0.03 mg/kg b.w.) for three weeks. The SO and second Orx group were treated s.c. with vehicle alone. A peroxidase-antiperoxidase (PAP) method was applied for localization of CT in the C cells. The volumes of C cells (Vc) and their volume densities (Vv) were determined using the multipurpose M_{42} test system. An ImageJ public domain image processing program was used to measure bone histomorphometric parameters of the proximal tibial specimens. Blood serum samples were analyzed for CT and osteocalcin (OC), and urine samples for Ca concentration. We found a significant decrease in the Vc, Vv, and serum CT in Orx rats compared with the SO. Tamoxifen treatment significantly increased Vc and serum CT compared to the Orx. Analysis of trabecular microarchitecture of tibia showed that Orx induced cancellous bone loss and marked decreases of cancellous bone area (B.Ar), trabecular thickness (Tb.Th), and trabecular number (Tb.N) whereas trabecular separation (Tb.Sp) was significantly increased. Serum OC and urinary Ca concents was considerably higher in Orx rats in comparison with SO. Administration of tamoxufen significantly enhanced B.Ar, Tb.Th and Tb.N and reduced Tb.N. Serum OC and urinary Ca concentrations were significantly lower than in the Orx group. These

findings indicate that tamoxifen stimulated calcitonin-producing thyroid C cells and increased trabecular bone mass in rat model of male osteoporosis.

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P82

The value of PRP/PRF in the treatment of bisphosphonates-induced maxillary osteonecrosis: preliminary study

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Background
Intravenous bisphosphonates treatment brings real benefits to patients with bone metastases. Unfortunately, this treatment sometimes has a difficult to treat complication, often disabling for the patients – the osteonecrosis of the jaws. A new approach in the management of this complication is the use of platelet rich plasma (PRP).
Patients and methods
Five patients aged between 62 and 72 years old (three men and two women) were included. All patients were treated with intravenous zolendronic acid (Zometa) for bone metastases (prostate cancer, breast cancer, hepatocarcinoma, or without a known starting point) or for multiple myeloma. When presenting, all patients had intraoral protruding mandibular and or maxillary bone sequesters. In all patients surgical debridement was accomplished, followed by covering with platelet rich fibrin (PRF) and a mucoperiosteal flap sutured in place. Both under the flap and in the surrounding gingival fibromucosa, PRP was injected. Two patients had osteonecrosis also in other areas of the jaws, presenting oral fistulas and radiographic evidence. In these cases PRP was injected in the fibromucosa adjacent to the affected area. Both PRF and PRP were prepared and used during the surgical procedure, from the blood of the patients harvested in tubes with separator gel with anticoagulant (PRP) or with procoagulant (PRF) and centrifuged at 3500 rounds/min 7.5 min.
Results
The intraoral postoperative wounds was completed healed within 10 days as well as the mucosal fistulas. In only one case a partial wound dehiscence with bone exposure was registered and healing was achieved by secondary intention.
Conclusions
Although the number of patients in the study is small, the first results of using PRP/PRF in the treatment of bisphosphonates induced jaw osteonecrosis are encouraging, both for treating the bone sequester when present, and to avoid its formation.

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P83

Bone mineral density in patients with hypersomatotropism

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Background and aim
Hypersomatotropism is known to be associated with musculoskeletal disorders, particularly, osteoporosis and increased fracture rates. However controversial data about the effects of acromegaly on bone mineral density (BMD) have been published. The aim of our research was to evaluate bone mineral density (BMD) in patients with hypersomatotropism.
Materials and methods
The study included 66 patients (20 men, 44 women) with hypersomatotropism (mean age 52 ± 11.37 years, BMI 29.64 ± 4.87 kg/m^2). In 64% (42 cases) acromegaly was caused by pituitary macroadenoma, in 36% (24 cases) by pituitary microadenoma. Serum GH and IGF1 concentration were elevated in all patients (6.7 ± 4.3 ng/ml; 475.9 ± 209.1 ng/ml accordingly). Control group consisted of 31 healthy volunteers (10 men and 21 women) matched for age and antropometric data (mean age $50.72 + 10.23$ years; BMI $29.38 + 4.81$ kg/m^2). Bone mineral density (BMD) was evaluated by dual X-ray absorbtiometry (DXA).
Results
We observed statistically significantly lower BMD values of the lumbar spine in patients with hypersomatotropism than in control subjects (1.19 (0.09–1.37) vs 1.32 (1.26–1.37) g/m^2, $U = 938.5$; $P = 0.009$). In the group of patients with hypersomatotropism older than 50 years osteoporosis was revealed in 10.9% (five patients); osteopenia – in 23.9% (11 patients) predominantly at lumbar spine (-0.08 ± 1.53 vs 0.21 ± 1.03; $P < 0.05$). In the group of 20 young age participants (men under 50 and women of reproductive period) low bone mass was registered at one patient (5%).
Conclusion
The results demonstrate a high prevalence of low bone mineral density at the spine in men older 50 years and postmenopausal women with acromegaly. Further investigation might be needed to predict risk factors of osteoporosis in patients with hypersomatotropism.

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P84

Effects of treatment for acromegaly on bone mineral density: is pegvisomant protective on lumbar BMD?

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Background
GH–IGF1 status is important for bone health. Acromegaly affects bone status, but less is known on the role of treatments for acromegaly on bone mineral density (BMD). Pegvisomant (Peg) is effective in treating acromegaly by reducing IGF1. As serum GH is not influenced by Peg, it is not known if residual, direct GH effects on bone (not IGF1 mediated) are preserved during treatment.
Methods
To evaluate the effects of Peg on BMD, we compared five patients treated with Peg (alone or in combination) to six patients treated with somatostatin analogues (SA) and to seven patients surgically cured, not under medical therapy. All the patients had normal serum IGF1. BMD was measured by DEXA (Hologic-QDR-2000 densitometer, Inc., Waltham, MA). A t-score of ≤ 1 and ≤ 2.5 at lumbar spine (L1–L4) and at femoral neck was used for diagnosis of osteopenia and osteoporosis, respectively.
Results
Mean age of subjects (seven males and nine females) was 60.7 ± 9.8 years. At lumbar spine, 40% of Peg-patients, 33.3% of SA-patients, and 60% of not-treated patients had osteopenia; none of the Peg-patients, and 16.7% of SA-patients, and none of not-treated patients were osteoporotic. Considering the femoral neck, 60% of Peg-patients, 33% of SA-patients, and 60% of not-treated patients had osteopenia; 20% of Peg-patients and none of the other two groups were osteoporotic.
Conclusions
The percentage of osteoporotic/osteopenic acromegalic patients seems to be lower than that reported in literature. Peg seems to protect bone at lumbar spine, but this protective effect does not seem to be exerted at femoral level where, indeed, patients treated with Peg present lower densitometric values. Patients surgically cured, not under medical therapy, have higher rate of lumbar osteopenia. No data are available on bone quality, a parameter that is usually altered in acromegaly.

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P85

HLA class I, Cw*01 and Cw*15 alleles can play a preventing role in serum IL17 elevation associated with postmenopausal osteoporosis in Hungary

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IL17 proinflammatory cytokine through T cell activation has prominent bone-wasting effect. Our previous results highlighted the role of IL17 in postmenopausal osteoporosis. To demonstrate the HLA allele association with the increased IL17 levels in postmenopausal osteoporosis was the object of the study.
HLA class I and II alleles were measured in 64 postmenopausal women (in four subgroups: with low IL17 (1, 3) and high (>3.04 pmol/l) IL17 levels (2, 4) with osteopenia and osteoporosis) by PCR technique. IL17 levels and bone mineral density (BMD) with *T*-score values in lumbar region (osteopenia: >-1 and <-2.5; osteoporosis: <-2.5) were detected with ELISA and dual energy X-ray absorptio-metry (DXA) using Hologic Discovery Wi, respectively. Allele frequencies on five loci were calculated with Arlequin software and 2×2 crosstabs for χ^2, and Fischer's exact tests were performed with SPSS Software.
The IL17 levels were as follows in osteopenic subgroups (1 and 2): 2.91 ± 0.12 ng/ml, $n=8$ and 3.48 ± 0.38 ng/ml, $n=15$; in osteoporotic subgroups (3 and 4): 2.85 ± 0.12 ng/ml, $n=6$ and 3.79 ± 0.55 ng/ml, $n=35$). The results gave significant difference in Cw*01 and Cw*15 HLA class I allele frequencies (AF%) between osteoporotic women with high (4) and low (3) IL17 levels (AF% 1.4 vs 25%, $P<0.009$ for Cw*01 and AF% 0 vs 16.67%, $P<0.02$ for Cw*15 by Fischer exact test).
In conclusion, our findings suggested that increased IL17 levels can accelerate the bone-wasting in postmenopausal osteoporosis. Postmenopausal osteoporotic women with increased frequency of Cw*01 and Cw*15 HLA alleles showed significant lower IL17 levels compared with those who had these alles in decreased frequency on C locus. The presence of Cw*01 and Cw*15 HLA class I alleles played preventing role in the increase of IL17 in hungarian osteoporotic women.

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P86

Bone health in type 1 diabetes patients with celiac disease

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Objectives
Type one diabetes mellitus (T1DM) is associated with various autoimmune conditions including celiac disease. Both these conditions are independently and variably associated with risk of osteoporosis. The current study intended to study bone health parameters and factors affecting it in patients with T1DM with serological evidence of celiac disease (CD).
Methods
A cross sectional study including 100 type one diabetes patients following up in our hospital was screened for CD bye IgA tissue transglutaminase (TTG) levels. Twelve patients (12%) patients tested positive. Twenty age- and sex-matched T1DM (IgA TTG negative) patients served as controls. After history and physical examination, biochemical parameters including serum levels of ionized calcium, inorganic phosphorus, alkaline phosphatase, parathyroid hormone and 25 hydroxy vitamin D were measured. Bone mineral density (BMD) were measured at total body (TB), lumbar spine (LS) and left femoral neck (FN) using dual energy X-ray absorptiometry (Lunar DRX DPO). Similarly DXA scan was done for measurement of total body bone mineral content (TBBMC), bone area (TBBA) and body composition. All the parameters were expressed as mean $\pm$ S.D. Data were analyzed using online graphpad quickcalc software and $P<0.05$ was considered statistically significant.
Results
TBBMD (0.77 ± 0.04 vs 0.81 ± 0.05 gm/cm^2) and TBBMC (801 ± 143 vs 982 ± 196) were lower in type one diabetic subjects with IgA TTG positivity ($P<0.05$). Similarly the total body *Z*-score (-1.64 ± 0.56 vs -0.46 ± 0.67), lumbar spine *Z*-score (-1.42 ± 0.61 vs -0.22 ± 0.83) and femoral neck *Z*-score (-1.48 ± 0.52 vs -0.34 ± 0.79) and TBBMC for age *Z*-score (-1.3 ± 0.8 vs -1.0 ± 0.9) were lower in type one diabetic subjects with IgA TTG positivity ($P<0.05$). However, TBBA (1038 ± 149 vs 1134 ± 156 cm^2) and TBBA for age *Z*-score (-0.9 ± 0.9 vs -0.8 ± 0.9) did not significantly differ between the two groups.
Discussion
Celiac autoimmunity is associated with reduced bone mineralization in T1DM patients. Celiac disease should be considered as a possible secondary cause of osteopenia in type one diabetic patients found to have a reduced BMD.
Conclusion
Important impact of early identification of CD in T1DM could be to prevent this important complication.

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P87

Oxidative stress in middle age males with osteoporosis: correlation of hormonal pattern and plasma total antioxidant capacity

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Male idiopathic osteoporosis is an underestimated disease, despite its clinical and social importance. The biochemical mechanisms are still poorly understood even if the interaction between genetic factors and hormone environment (especially gonadal steroids and GH) plays a undoubtful role. In previous studies we demonstrated low plasma total antioxidant capacity (TAC) in hypogonadal patients. The aim of this study was to investigate oxidative stress as risk factor for bone fracture, and its relationships with endocrine milieu.
We enrolled 31 male subjects (36–72 years), all affected by back pain/spine fracture as a consequence of trivial trauma and ten healthy controls (30–48 years). TAC was determined using a colorimetric assay, using the system H_2O_2–metmyoglobin as source of radicals and a chromogen (ABTS); the latency time (LAG) in the accumulation of $ABTS^{\cdot+}$, spectroscopically detectable, is proportional to antioxidants concentration. An endocrine evaluation including testosterone, estradiol, insulin, IGF1, PRL, FT_3, FT_4, TSH levels was also performed. Finally, bone mineral density was assessed by DEXA. Bone metabolic parameters were evaluated (PTH, vitamin D, osteocalcin, and β-cross laps). Statistical evaluation was performed using Mann–Whitney *U* test.
The prevalence of IGF1 defects (52.8 ± 15.28 ng/ml) was 5/31 (suggesting GH deficiency (GHD), confirmed by GHRH+arginine test). Hypogonadism (mean testosterone levels 2.03 ± 0.46 ng/ml) was present in 4/31. The 22 patients left did not show alterations in the hormonal parameters studied. Despite mean levels of LAG were not different between patients and controls (72.7 ± 8.5 vs 75.0 ± 6.0 s), 12 out of 31 patients had low LAG levels (between 50 and 60 s) irrespective of hormonal milieu. Moreover, when considering parameters of bone metabolism we found significantly lower vitamin D levels in hypogonadal subjects, than in patients with GHD and patients with normal hormonal parameters (10.7 ± 5.8 ng/ml vs 19.7 ± 17.7 and 22.7 ± 9.7 respectively).
These preliminary data suggest a possible involvement of oxidative stress in unexplained fractures even if further investigations are needed to establish a possible correlation with anabolic hormones involved in bone metabolism. Low vitamin D levels could exert a worsening effect on osteoporosis in hypogonadal patients.

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P88

Lean body mass and leptin, but not fat mass are independent predictors of bone mass in postmenopausal women

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Body weight is positively correlated to bone mass. While gravity represents a stimulation stress for bone turnover, endocrine function of adipocytes may also influence bone.
Objectives
We investigated the relative influence of weight, body composition and leptin upon lumbar bone mass in pre and postmenopausal women.
Materials and methods
Cross-sectional study including six groups varying from 8 to 15 pre- / postmenopausal healthy volunteers with different weights (lean BMI <25 kg/m^2, overweight BMI 25–30 kg/m^2, and obese BMI >30 kg/m^2), not exposed to antiosteoporotic therapy. Lumbar bone mineral density (BMD) and body composition (BC) were evaluated by dual X-ray absorptiometry (DXA, Hologic), while serum leptin was evaluated by ELISA.
Results
Lean and overweight postmenopausal women had lower lumbar BMD than premenopausal women ($P<0.05$).
Bone mass of obese postmenopausal women did not differ from that of premenopausal women. Body weight and compartments were all positively correlated to bone mineral content. The best correlation was attained with lean

mass ($r^2 = 0.47$ for the whole group), that was an independent predictor of bone mass irrespective of age ($P < 0.05$, multivariate analysis, ANOVA). Leptin was an independent predictor for bone mass only for postmenopausal women ($P < 0.05$).
Conclusion
Increased body weight prevents bone loss after menopause. Lean mass predicts bone mass independently of body weight irrespective of age. The beneficial role of fat mass and total body weight on bone mass through gravitational stress seem to be supplemented by possible direct effects of leptin on bone at postmenopausal women.

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P89

Association of the (TTTA)*n* repeat polymorphism of CYP19 gene with bone mineral density in Greek peri- and postmenopausal women

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Introduction
Aromatase is encoded by the CYP19 gene and catalyzes the conversion of androgens to estrogens, which in turn regulate skeletal homeostasis. Polymorphisms in the CYP19 gene have been studied for their association with bone mineral density (BMD) in the general population with mixed results.
Objectives
To explore the influence of the CYP19 (TTTA)*n* repeat polymorphism on BMD and serum levels of osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) and bone metabolic markers in a Greek female population.
Methods
Two hundred and seventeen peri- and postmenopausal women aged 42–63 years were enrolled. All participants underwent spinal BMD evaluation by dual-energy X-ray absorptiometry (DXA). Genotyping of the (TTTA)*n* repeat polymorphism was performed by PCR. Levels of OPG, soluble RANKL (sRANKL) and bone metabolic markers were measured.
Results
Genotype analysis revealed alleles having 7–12 TTTA repeats. Women carrying the $(TTTA)_{11}$ and/ or $(TTTA)_{12}$ alleles had significantly higher spinal BMD than women not carrying these alleles in both the total study population as well as in the subgroup of women with osteoporosis ($P = 0.042$ and $P = 0.006$ respectively). The aforementioned associations remained significant after adjustment for age, years since menopause, smoking and BMI ($P = 0.048$ and $P = 0.023$, respectively by multivariate analysis). No association of the (TTTA)*n* polymorphism with circulating levels of OPG, sRANKL, and bone metabolic markers was observed.
Conclusions
The (TTTA)*n* polymorphism of the CYP19 gene influences BMD at the lumbar spine in peri- and postmenopausal Greek women and is potentially involved in the regulation of bone metabolism.

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P90

Vitamin D receptor polymorphisms and bone mass indices in post menarcheal indian adolescent girls

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Objective
The aim of the present study was to assess the association between vitamin D receptor (VDR) gene polymorphism and bone mass indices in Indian adolescent girls.
Methods
The current study was a cross sectional one including 100 post menarcheal girls aged 15–18 years. Serum levels of ionized calcium, inorganic phosphorus, alkaline phosphatase, parathyroid hormone and 25 hydroxy vitamin D were measured. Bone mineral content (BMC), Bone area (BA) and bone mineral density (BMD) were measured at total body (TB), lumbar spine (LS) and left femoral neck (FN) using dual energy X-ray absorptiometry (Lunar DRX DPO). Polymorphisms of VDR gene at the Fok1 and Bsm 1 loci were detected using SYBR Green quantitative PCR.
Results
Vitamin D deficiency (serum 25-OH D3 < 30 ng/ml) was observed in 43% patients. The overall prevalence of genotype for Bsm1 in this study was 33.3% Bb, 29.2% bb and 37.5% BB. For Fok1 genotype, the prevalence was 44.2% Ff, 7.5% ff and 48.3% FF. There were no significant differences in the blood parameters when classified according to Bsm1 and Fok1 genotypes. Subjects with BB genotype have significantly higher mean TBBMC (1012 ± 178 g), TBBA (1264 ± 186 cm^2), TBBMD (0.89 ± 0.06 g/cm^2) and LSBMD (0.81 ± 0.04 g/cm^2) than Bb and bb ($P < 0.05$). They showed tendency for association with LSBMC and LSBA ($P < 0.1$). Bsm 1 genotype did not show an association with FN bone indices whereas Fok1 genotype did not show an association with TB, LS or FN bone indices.
Discussion
Vitamin D is important for bone health. Vitamin D deficiency is common among children and adolescents in India, in spite of abundant sunshine. With respect to the Bsm1 genotype, the Bb and bb subgroups were more prevalent (62.5%) than BB (37.5%) and were associated with worse bone health parameters. Whereas with respect to the Fok1 polymorphism, FF genotype was most common (48.3%). But there was no difference in the bone health parameters among different subgroups.
Conclusion
The present study demonstrates VDR gene polymorphism; defined by Bsm 1 genotype has an influence on total body and lumbar spine bone mass indices in post menarcheal Indian girls.

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P91

Bone health in children with GH deficiency

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Objectives
The current study intended to assess the impact of GH deficiency (GHD) on bone health after using various size corrections.
Methods
Thirty prepubescent children with GHD (male:female = 20:10, mean age -9.4 ± 3.5 years) were included in the study. Data on anthropometry and total body bone mineral content (TBBMC), bone area (TBBA) and lean body mass (TBLBM) by dual energy X-ray absorptiometry were collected. Anthropometric *Z*-scores and bone parameter *Z*-scores were computed using ethnic normative reference database.
Results
Mean height for age *Z*-score (HAZ) was -5.1 ± 1.7. Mean TBBMC for age *Z*-score was -9.2 ± 6.3 and mean TBBA for age *Z*-score was -7.1 ± 4.3. All the study children had 'short bones' with HAZ < -2. Twenty four (80%) children had 'narrow bones' (TBBA for height *Z*-score < -2). Twenty one (70%) children had 'light bones' (TBBMC for TBBA *Z*-score < -2). Mean TBBMC for age *Z*-scores were significantly lower than the mean HAZ ($P < 0.05$), indicating lower BMC after adjusting for height. Mean TBBMC for TBLBM *Z*-score was -3.3 ± 4.2, indicating bone mineral deficit even after adjusting for TBLBM. There was no significant gender difference in any of the parameters.
Discussion
GHD in children causes low bone mineral density (BMD). Height and muscle force drive bone mineralization. International society of clinical densitiometry has made it obligatory to applying size corrections. Analysis of different bone health parameters lead to the demonstration that Indian children with GHD have 'short bones' (100% cases), 'narrow bones' (80% cases) and 'light bones' (70% cases).
Conclusion
Indian prepubertal GHD children had low bone mass even after applying size corrections implying need for corrective measures for their bone health.

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P92

The effect of monthly Ibandronate on bone mineral density and bone turnover markers in patients with hemophilia A or B and increased risk for fracture

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Introduction
Haemophilia A and B has been associated with increased prevalence of low bone mineral density (BMD). However, no study has so far evaluated the effects of anti-osteoporotic therapy on BMD.
Methods/design
The primary endpoint of this prospective study was to estimate the effect of 12-month therapy of oral ibandronate 150 mg/month on BMD in patients with haemophilia A and B. Secondary endpoint was its effect on bone turnover markers (BTM), including serum C-terminal telopeptide of type one collagen (sCTX) and tartrate-resistant acid phosphatase band 5b (as markers of bone resorption), osteocalcin and bone-specific alkaline phosphatase (as markers of bone formation). We present an interim analysis of the first 12 months of the study. Adult patients with T-score < -2.5 s.d. or Z-score < -2 and/or increased risk of fracture according to FRAX model were included. All received 1000 mg/day calcium carbonate with 800 IU/day cholecalciferol.
Results
Ten males (aged 43.7 ± 13.8 years, seven with haemophilia A) were included. Ibandronate resulted in a significant increase in lumbar BMD (from 0.885 ± 0.162 to 0.926 ± 0.177 g/cm^2, ($+4.9\%$), $P=0.011$). No significant change in BMD of total hip (from 0.717 ± 0.128 to 0.729 ± 0.153 g/cm^2, $P=0.963$) or neck (0.741 ± 0.135 to 0.761 ± 0.146 g/cm^2, $P=0.952$) was noticed.
Ibandronate led to a significant decrease in sCTX (from 0.520 ± 0.243 to 0.347 ± 0.230 ng/ml, -29.9%, $P=0.042$). No significant change in the other BTM was observed. Ibandronate was generally well-tolerated. No fractures were reported.
Conclusions
In the first study conducted so far evaluating the effect of bisphosphonates in patients with haemophilia and increased fracture risk, ibandronate significantly improved BMD in lumbar spine and reduced bone resorption. Its effect on hip BMD and bone formation markers was not significant.

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P93

Age overlooked as a risk factors for osteoporosis

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Osteoporosis is a debilitating condition that can be prevented by timely detection and treatment. Detection requires referral for dual energy X-ray absorptiometry (DXA) that depends on risk factors. We estimated the importance of risk factors for a T-score of < -2.5 measured by spine and hip DXA in all men and women referred from general practice for a DXA scan at Aalborg University Hospital during a 3 years period. Risk factors were assessed by questionnaire and referral papers. A total of 5335 women and 818 men were referred: 3042 aged 50–69 years/1726 aged 70 years or above/484 aged 80 years or above. A low T-score was found in 33.2/41.1/46.9% respectively ($P<0.001$). No risk factors were identified in 13.3/15.3/17.4% ($P=0.10$) when aged was excluded as a risk factor. Risk factor occurrence differed between the age groups 50-69/70-79/80+ years for family history (46/25/15%; $P<0.001$), predisposing disease (21/30/25%; $P<0.001$), steroid use (5.6/12.3/13.2%; $P<0.001$), low sun exposure (12.8/16.5/24%; $P<0.001$), smoking 25/19/10%; $P<0.001$), and high alcohol intake (5.8/5.1/2.0%; $P=0.004$), while not for premature menopause (21/20/15%; $P=0.12$), low intake of dairy products (4.3/4.4/2.5%; $P=0.18$), or low BMI (2.4/2.0/2.0%; $P=0.69$). Fragility fractures increased with age (women, 21/39/49%, $P<0.001$; men, 24/32/29%, $P=0.088$). In conclusion, the T-score and fracture dependence on family history, sun exposure, smoking, and alcohol intake was as expected. However, the limited change in risk factor occurrence with age suggests that age per se had too low impact on referral for DXA scanning, even though age is a major determinant of fracture risk.

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P94

Trabecular bone score and bone mineral density in Ukrainian normal women depending on duration of postmenopausal period

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Aim
The aim of this study is evaluating trabecular bone score (TBS) and bone mineral density (BMD) in normal women depending on duration of postmenopausal period (PMP).
Materials and methods
We've examined 122 normal women aged 40–79 years (mean age – 56.2 ± 0.8 years; mean height – 162.6 ± 0.5 cm; mean weight – 71.5 ± 1.3 kg), who were divided into the groups depending on duration of PMP: without menopause (with normal menstrual cycle) ($n=25$), 1–3 years ($n=26$), 4–6 years ($n=12$), 7–9 years ($n=18$), 10–12 years ($n=8$), 13–15 years ($n=4$), 16-18 years ($n=5$), more 19 years ($n=7$). BMD of total body, lumbar spine and femoral neck and TBS (TBS iNsight software package, Med-Imaps) were measured by DXA using a densitometer Prodigy, GE.
Results
We have determined the significant decrease of TBS (L1–L4) in women with age ($F=4.52$; $P=0.0001$). Duration of PMP has a significant influence on the variability of BMD of spine ($F=3.20$; $P=0.004$), BMD of femoral neck ($F=5.41$, $P<0.000$) and TBS (with normal menstrual cycle – 1.36 ± 0.02/mm, 1–3 years – 1.31 ± 0.02/mm, 4–6 years – 1.22 ± 0.04/mm, 7–9 years – 1.23 ± 0.03/mm, 10–12 years – 1.21 ± 0.04/mm, 13–15 years – 1.16 ± 0.08/mm, 16–18 years – 1.15 ± 0.06/mm, more 19 years – 1.15 ± 0.03/mm; $F=5.70$; $P<0.000$). The analysis using Scheffe's test shows that TBS was significantly lower in women with duration of PMP more 4 years (4–6 years ($P=0.003$), 7–9 years ($P=0.002$), 10–12 years ($P=0.002$), 13–15 years ($P=0.0003$), 16–18 years ($P=0.0003$), and more 19 years ($P=0.00002$)) in compare with women without menopause; BMD of spine significantly decreased in women with duration of PMP 7–9 years ($P=0.02$), 10–13 years ($P=0.003$), and more 19 years ($P=0.0001$).
Conclusion
TBS is independent parameter which has potential diagnostic value without bone mineral density. TBS was significantly decreased with age. Duration of PMP has a significant influence on the variability of TBS, BMD of spine and femoral neck. TBS significantly decreased in women in early postmenopausal period.

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P95

Bone metabolism with special emphasis to osteoprotegerin and vitamin D_3 concentrations in premenopausal women with thyroid dysfunction

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Objectives
The objective was to evaluate effects of hyperthyroidism and hypothyroidism on bone metabolism in premenopausal women with special emphasis to osteoprotegerin (OPG) and vitamin D_3 (vD).
Materials and methods
A total of 119 women aged 18–52 years were studied (38 with recently diagnosed hyperthyroidism; 40 with newly diagnosed hypothyroidism; 41 healthy women as controls). Patients were followed up for a period of 1 year. BMD, bone turnover markers and hormonal analyses with regard to seasonal changes in $25(OH)D_3$ were carried out at 0, 6 and 12 months.
Results
On the initial evaluation lower femoral neck BMD was found in patients with hyperthyroidism compared to hypothyroidism. Higher bone markers, osteocalcin (OC) and collagen type one crosslinked C-telopeptides (CTX) were noted in hyperthyroidism than in controls and hypothyroidism. A difference was not found in OPG nor vD among the studied groups. After 6-month in patients treated due to

hyperthyroidism, higher OC was demonstrated (vs hypothyroidism and controls) and CTX (vs controls). In this group a decrease in CTX was also demonstrated. No difference was shown in OPG among the studied groups. However, a statistically significant lower vitamin D_3 was demonstrated in he treated group with hypothyroidism and the control group. In the group with hyperthyroidism decrease of OC and CTX were observed. After 1 year increase in OPG was seen in the group treated due to hyperthyroidism and hypothyroidism.
Conclusions
A negative effect on bone metabolism is observed only in the group of female patients with hyperthyroidism. Osteoprotegerin seems not to be a useful marker of bone metabolism in thyroid dysfunction. Vitamin D_3 was significantly decreased, independent of the season, in all groups studied.

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Efficacy and safety of monthly oral minodronate for the treatment of glucocorticoid-induced osteoporosis in rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that results in generalized bone loss and increased fracture risk. Although glucocorticoids are frequently prescribed for the symptomatic management of inflammatory disorders such as RA, extended glucocorticoid exposure is the leading cause of osteoporosis and leaves patients at a high risk of fracture. The aim of this study is to evaluate the efficacy and safety of monthly oral minodronate for the treatment of osteoporosis induced by glucocorticoid and RA itself, and to compare minodronate with weekly or daily risedronate in glucocorticoid-induced osteoprotic RA patients. Minodronate was monthly administrated to 36 osteoporotic RA patients at a dose of 50 mg for 24 weeks. Ten RA patients were treated with no bisphosphonate (group I) before minodronate treatment. In contrast, 16 or 10 RA patients were treated with weekly or daily risedronate (groups II or III), respectively, and changed to monthly minodronate. Lumber and total hip bone mineral density were measured at 0 and 24 weeks. Serum tartrate-resistant acid phosphatase 5b (TRAP-5b) and bone-specific alkaline phosphatase (BAP), parathyroid hormone (PTH), and urinary type-I collagen cross-linked-N-telopeptide (uNTx) were measured. RA activity and a mean dose (7.3 + 1.1 mg/day) of oral prednisolone were not significantly changed in three groups. The percentage change from baseline (0 week) in lumber and total hip bone mineral density was increased in group I and III at 24 weeks. In contrast, bone mineral density in group II at 24 weeks was not changed compared with baseline (0 week). In addition, TRAP-5b and uNTx at 24 weeks were significantly decreased in group I and III ($P<0.01$) and BAP was weakly decreased in all groups at 24 weeks. Moreover, serum concentration of PTH was increased in group I, but was reduced in group II and III. In addition to those data, serum calcium was decreased in group II and III, but not group I. In conclusion, monthly administration of minodronate was effective in glucocorticoid-induced osteoporosis in RA patients. Furthermore, monthly minodronate was thought to be strong bisphosphonate, as compared with daily risedronate. Especially, the effect of minodronate on the induction of secondary hyperparathyroidism might be weak as compared to that of risedoronate.

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P97

Change in bone mass in patients with established hyperthyroidism

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Objectives
To evaluate the presence and the degree of osteoporosis and osteopenia in a group of patients with established hyperthyroidism from at least 6 months.
Material and methods
This study was based on a quantitative measurement of bone mineral density with and heel ultrasound densitometer (Type Pegassus). Each selected patient was recorded for the weight, height, BMI, age, and gender. No patient was previously treated for osteoporosis or osteopenia. A control group with similar data was selected form the general population, with no personal or familiar history of hyperthyroidism. None of them had a known history for osteoporosis or had received any medication for this condition. The criteria for osteoporosis were those recommended from the WHO. Osteoporosis $= T$-score < -2.5.
Results
We studied 64 patients with confirmed hyperthyroidism from at least 6 months and 38 persons of the same age and gender in healthy conditions. There were 22 males and 42 females (33/67%). Mean age 59.9 ± 12.1 years, weight 74 ± 2 kg, BMI 27.8 kg/m^2. For the control group: 38 patients (14/24 M/F), age 60.5 ± 11.1 years, weight 69.9 ± 12.3 kg, BMI 26.2 kg/m^2. The mean values of T-score for the hyperthyroid patients were -3.7 ± 1.4 and -2.0 for the control group. 55% of patients with hyperthyroidism had severe osteoporosis, compared with only 9.5% of control group ($P<0.001$). The gender itself was no significant.
Conclusions
The silent osteoporosis and osteopenia is relatively frequent in hyperthyroidism, significantly more than in normal population. The stimulation of osteoclasts more than osteoblasts and alteration of remodeling cycling from thyroid hormones is believed to be the causative factor.

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P98

The follow-up in patients evaluated based on quantitative ultrasound: a prospective study

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Introduction
The heel quantitative ultrasound (QUS) is useful tool in the assessment of fracture risk especially if cost issues are involved. It might be helpful in follow-up the patients who will not be treated with anti-osteoporotic drugs.
Aim
To present the bone evaluation (DXA) after a period of time in patients who are initially assesed by both DXA and QUS.
Material and patients
This is a prospective pilot study in a group of postmenopausal women. They were not treated with anti-resorbtive drugs at first evaluation, meaning central DXA (at least two sites) and heel QUS (GE Achilles). After at least one year they were re-evaluated only by DXA (because of data lack in QUS use in patients treated with anti-osteoporotics as the patients initially diagnosed with osteoporosis started therapy).
Results
Out of 360 patients who were first evaluated, 61 patients were followed-up for mean 23.53 months. The initial mean age was 55.77 years. We formed two subgroups of patients based in QUS stiffness index (SI). Lower SI than 79 U (meaning high fragility fracture risk) includes a group of 29 patients (mean SI of 95.61 U, and higher SI than 79 U (meaning low fracture risk) includes 32 patients (control group) with a mean SI of 61.9 U. The number/percent of patients in each group with osteoporosis/osteopenia/normal DXA were in studied group: 5 patients/17.24%, 16 patients/55.17%, 8 patients/27.58%; in control group 7 patients/21.8%, 10 patients/31.25, and 15 patients/46.87%. The patients were followed-up for 23.78 months in group 1 vs 22.81 months in group 2. The second DXA evaluation showed in the first group 20.68% of patients had significant changes in bone mineral sensity (16.66% of these had higher BMD and the others lower BMD), while only 9.3% of women from the control group had significant changes in BMD (all higher BMD).
Conclusion
In patients with high fragility fracture risk based on QUS, the ~ 2 years follow-up period of time pointed a higher number of patients with significantly BMD decreased than the group with low fracture risk. This is an argument that the DXA and QUS are two complementary methods in fracture risk evaluation.

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Prospective study of bone mineral density in girls treated with estroprogestagens for functional hypothalamic amenorrhea during late puberty in relation to the polymorphism of VDR (*BsmI*), ER (*PvuII; Xba I*), and COLIA1 genes

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Introduction
The aim of this study was to verify if genetic factors influence the short- and long-term therapeutic responses to oestroprogestagen (OP) therapy, implemented in girls with functional hypothalamic amenorrhoea (FHA) in order to improve their bone mineral density (BMD).
Material and methods
The study included 84 FHA girls who underwent a 4-year sequential OP therapy with 17-β oestradiol and didrogesterone. Changes in the lumbar spine BMD were determined at the end of the therapy and 6 years after its discontinuation, and analysed in regards to PvuII and XbaI polymorphisms of oestrogen receptor-alpha gene, BsmI polymorphism of vitamin D receptor gene, and Sp1 polymorphism of the type-1 collagen gene.
Results
After 4 years of OP therapy, a significant increase in BMD was documented in the studied group. Follow-up densitometry performed 6 years after completing the therapy revealed a significant decrease in BMD level; nonetheless, the values of this parameter were still significantly higher compared to pretreatment level. Neither the particular polymorphisms nor their combinations influenced the relative change in BMD at the end of the therapy and after a 6-year follow-up.
Conclusions
Variability of genes involved in oestrogen, vitamin D and collagen metabolism does not influence the short- and long-term results of OP therapy in girls with FHA.

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P100

Vertebral fracture prevalence in postmenopausal women in relation to bone mineral density (BMD)

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Introduction
Epidemiological studies show that one half of vertebral fractures, which play an important role in estimation of future fracture risk, remain unrecognized.
Aim
To establish vertebral fracture frequency in relation to bone mineral density in postmenopausal women.
Methods
Study comprised 146 postmenopausal women. On the grounds of spine and hip DXA *T*-score based on WHO criteria subjects were divided to healthy, osteoporosis or osteopenia. Vertebral fractures were verified by lateral DXA scan vertebral morphometry of thoracic and lumbar spine.
Results
Vertebral fractures were found in 111 (76%) women; 58 (52.2%) in osteoporosis, 33 (29.7%) in osteopenia and 20 (18.1%) in healthy subjects. Statistically significant difference was found between age of women with osteoporosis compared to group with osteopenia and healthy group (67.86 ± 9.24 vs 62.97 ± 8.74 years; 67.86 ± 9.24 vs 61.55 ± 10.53 years.; $P < 0.05$). Statistically significant difference between women in relation to body mass index was not found. Thoracic vertebra fractures dominated in all three groups with similar frequency of compressive and second degree wedge fractures. Statistically significant higher frequency of vertebral fractures was found in group with osteoporosis compared to healthy osteoporosis group ($P < 0{,}001$). Statistically significant higher frequency of lumbar vertebre fractures was seen in osteoporosis compared to osteopenia and healthy subjects. Higher frequency of vertebral fractures in women with osteopenia compared to healthy group was not statistically significant.
Conclusion
Lateral DXA scan can be used in estimation of fracture risk in addition to BMD, clinical and biochemical risk factures to provide better insight in individual fracture risk and help decide upon activities and therapy in fracture prevention.

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P101

Vertebral fractures in women with postmenopausal osteoporosis with or without prior fragile fracture

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Introduction
Vertebral fragile fracture is risk factor for future vertebral and hip fractures.
Aim
To establish prevalence of vertebral fractures using lateral DXA scan of thoracic and lumbar spine in women with postmenopausal osteoporosis.
Method
Study comprised 73 women with postmenopausal osteoporosis diagnosed using spine and hip DXA. Vertebral fractures were verified by lateral DXA scan vertebral morphometry of thoracic and lumbar spine.
Results
37 (50.7%) women had prior non-vertebral fragile fracture (group A) and 36 (49.3%) did not (group B). Group A had fractures as follows: 26 (70.3%) distal radius, 2 (5.4%) hip, 3 (8.1%) ribs, 2 (5.4%) proximal humerus, 4 (10.8%) distal fibula. There was not tatistically significant difference in age and BMI between two groups (67.24 ± 7.81 vs 67.58 ± 10.28 years; BMI: 26.42 ± 3.94 vs 24.76 ± 3.94 kg/m^2). Average *T*-scores in group A were: L1–L4 -2.5 S.D., femur neck -1.83 S.D., hip -1.94%. In group B: L1–L4 -3.2 S.D., femur neck -2.3 S.D. and hip -2.27 S.D. There was statistically significant difference of L1–L4 *T*-score among groups (group A -2.5; group B -3.2; $P < 0.005$). In group A 7 (18.9%) women did not have vertebral fracture, 9 (24.3%) one, 21 (56.7%) two or more and 7 (18.9%) five. In group B 8 (22.2%) had no vertebral fractures, 8 (22.2%) one, 20 (55.5%) two or more, and 11 (30.5%) had two vertebral fractures. In both groups thoracic vertebrae fractures of second degree were more frequent, with similar frequency of compressive and wedge fractures. Women in group A had statistically significant higher frequency of vertebral fractures ($P < 0.05$) and lumbar vertebrae fractures ($P < 0.05$).
Conclusion
Women with prior fragile fracture have greater frequency and more vertebral fractures, with lumbar vertebrae fractures with repercussions on BMD. Lateral DXA scan helps in spine and hip DXA BMD interpretation and individual fracture risk estimation.

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P102

Effect of long-term valproate therapy on bone health parameters

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Introduction
Anti-epileptic drugs (AED) adversely affecting bone health. However, individual variations between different AEDs exist and not all AEDs are equally toxic to the bone. There is almost equipoise about the effect of valproate on bone.
Methods
We conducted a cross-sectional study assessing bone mineral density with dual energy X-ray absorptiometry at the hip and lumbar spine in 33 outpatients receiving valproate for ≥ 1 year and 36 healthy adults. Plasma total calcium, phosphate, intact parathyroid hormone, total alkaline phosphatase and 25 hydroxy vitamin D were determined in both groups. All participants were evaluated for daily dietary calcium intake by dietary recall method.
Results
Patients had taken 1020 ± 575.06 g (cumulative dose) of valproate over 6.46 ± 3.83 years of epilepsy. The mean ($\pm$S.D.) bone density in patients, as compared to

controls, treated with antiepileptic drugs was 0.987±0.121 vs 0.940±0.094 g/cm^2 at the spine and femoral neck 0.858±0.146 vs 0.818±0.136 g/cm^2. Difference between two groups was not significant statistically. Osteopenia was present in 90.62% of controls and 85.71% of controls. Osteoporosis was present in 6.25% of patients and 14.29% of controls. Serum intact parathyroid hormone was significantly higher in patients (60.32±29.16 vs 45.16±20.00 pg/ml, $P=0.01$), but the total calcium, 25-hydroxy vitamin D, alkaline phosphatase and dietary intake of calcium did not differ significantly between patients and controls. But, both groups displayed grossly low serum vitamin D (patients: 12.44±9.91 ng/dl, controls: 13.87±8.57 ng/dl) and dietary calcium intake below recommended daily allowance.

Conclusion

Our results suggest that chronic therapy with valproate does not result in significant decrease in bone density. Widespread vitamin D deficiency is still prevalent in our country.

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P103

Efficacy and safety of the anabolic therapies in severe osteoporosis: experience of a team of endocrinologists and spine surgeons

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Anabolic therapies represent a major advance in the management of severe osteoporosis. Parathyroid hormone (PTH) and human recombinant PTH peptide 1–34 (Teriparatide) demonstrated an increase in bone mineral density and a significant reduction in vertebral fractures in patients with osteoporosis when given for 18–24 months. The intermittent administration of PTH or teriparatide stimulates osteoblastic function, improves bone architecture and has an additional analgesic effect.

We retrospectively analyzed the safety, efficacy and adherence to therapy with anabolic agents given for 18 months in 79 patients (F/M: 72/7) with severe osteoporosis resistant to antiresorptive therapy. Patients were followed by a team of spine surgeons and endocrinologists from 2007 to 2012. Before anabolic therapy 40 patients underwent percutaneus kyphoplasty, 23 were treated with brace and 16 patients refused any orthopaedic treatment. Anabolic agents were administered as a daily injection, and vitamin D supplementation was given, when necessary. The change in BMD value was measured at the beginning of therapy and after 18 months through lumbar and femoral DEXA scan.

A total of 64 patients completed the 18 months treatment with anabolic agents. 15 patients (19%) discontinued the treatment: 11 patients because of side effects occurring during the first 3 months of therapy (hypercalcemia occurred in eight patients) and four patients due to non adherence to therapy. All patients had vertebral fractures (VF) (mean number of VF 3.2±1.4) and suffered from back pain. During anabolic therapy, 31% of patients showed an improvement of back pain. At the end of the treatment, there was a substantial increase in BMD at both lumbar spine (+8.5%) and femoral neck (+5%).

This study confirms that anabolic agents are safe and effective. Adherence to therapy is very high, despite the daily administration. Of note, this therapy reduce back pain in a significant percentage of patients.

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P104

Vitamin D receptor, BsmI, FokI, ApaI, TaqI and estrogen receptor alpha, PvuII and XbaI, gene polymorphisms in women with osteoporosis

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Objective

The aim of this study was to determine the frequencies of vitamin D receptor (VDR) Bsm1, Fok1, ApaI, TaqI and estrogen receptor α (ESR1) PvuII and XbaI polymorphisms in Romanian patients and to investigate their involvement in postmenopausal osteoporosis.

Methods

The study was carried out on 82 postmenopausal women, aged over 60, divided into two groups: group 1-postmenopausal women with osteoporosis (34 subjects), group 2-postmenopausal women without osteoporosis (48 controls). The hematological and biochemical profiles were evaluated. ESR1 (*XbaI* and *PvuII*) and VDR (*Bsm I, ApaI, TaqI* and *FokI*) polymorphisms were determined by PCR-RFLP method on genomic DNA.

Results

Bsm1, *Fok1, Apa I* and *Taq I* allele distribution were in Hardy Weinberg equilibrium with no significant differences between group 1 and 2. There were significant differences between distributions of *PvuII*, IVS-1 −397 T/C and *XbaI*, IVS-1 −351 A/G genotypes in osteoporosis group compared to healthy women. G allele of *XbaI* polymorphism seems to be a risk allele for osteoporosis ($P=0.03$, OR=7.6). No association was found between BMD and VDR polymorphisms.

Conclusions

The genotype and allele frequency distributions were in Hardy–Weinberg equilibrium excepting ESR1, *Xba I*, G allele that seems to be a risk allele for osteoporosis. VDR polymorphisms showed no significant difference between osteoporosis and control group.

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P105

Possible benefits of PTH 1–84 therapy in pregnancy and lactation osteoporosis (PLO)

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Introduction

Pregnancy and lactation associated osteoporosis (PLO) is a rare condition in which women typically present with fractures, often vertebral, in the third trimester of pregnancy or in the early *postpartum* period. Bone loss of 3–10% at the spine and hip are seen over 3–6 months of lactation. Bone loss is related to duration of lactation and amenorrhea and is not prevented by calcium supplementation.

Methods

We studied a woman of 45 years, in the third trimester of pregnancy, who suffered from constant low back pain, which prevented her to walk properly. Three months after childbirth, she underwent by DXA severe osteoporosis characterized by multiple vertebral fractures. Bone markers as alkaline phosphatase, osteocalcin, serum β-cross laps, PTH, 25OHD, 24 h urine calcium were assessed. Secondary causes of osteoporosis were also studied by specific laboratory tests: TSH, ematology, renal and epatic function, proteins, CA125, and CA15-3. All of them were considered within normal range. Patient suffered from reflux and esophagitis. Osteogenesis imperfecta were also excluded. We choose to treat her with osteoanabolic PTH 1–84 therapy.

Results

DXA showed increase in BMD at lumbar site after 18 months of PTH 1–84 treatment. Biochemical parameters increased at the 6th and the 12th month and then reducted, as described in the literature, at the 18th month of therapy. During this period the patient showed marked improvement in the lumbar algodistrofia, being able to walk slowly.

Conclusion

As this disorder is likely to be heterogeneous in its etiology and prognosis, a through evaluation for secondary causes of osteoporosis should be undertaken in all cases of PLO. Results obtained in our clinical experience confirm the efficacy of PTH 1–84 therapy on high risk of fracture in PLO, documented increase of *T*-score at the spine, besides the increase of β-Ctx and osteocalcin levels.

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P106

Influence of some traditional risk factors for osteoporosis on bone metabolism during substitution therapy of primary hypothyroidism

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Relation between thyroid hormones and bone metabolism markers in hyperthyroidism is well known. Earlier studies indicate possibility of bone metabolism acceleration during the excessive replacement therapy with L-thyroxine in hypothyroid patients especially in one with other risk factors for bone metabolism impairment. This study evaluated the effect of physiological L-thyroxine treatment on bone metabolism in patients with primary hypothyroidism.
In a study group of 30 hypothyroid patients individual L-thyroxine replacement was performed targeting euthyroid status. Bone and calcium metabolism parameters (osteocalcin-OC, alkaline phosphates-ALP, C-terminal cross-linking telopeptide type I-CL, parathormone-PTH, Ca, ionized Ca, P), thyroid hormone levels (T_3, T_4, TSH) were measured before treatment and when euthyroid status was achieved.
In this study the following parameters were examined: influence of BMI, physical activity, time of menarche, duration of lactation period, number of children and occurrence of menopause before therapy and when the euthyroid status was achived on bone metabolism markers.
Significant difference by dispersive analysis was found in bone metabolism markers concerning influence of menopause, physical activity and BMI in treated hypothyroid patients. We did not found significant difference in bone metabolism markers among groups concerning parameters like duration of lactation period or number of live born children.
Key Words
Hypothyroidism, bone and bone metabolism, L-thyroxine substitution therapy, risk factors for osteoporosis.
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P107

Resolution of anaemia after curative parathyroidectomy in a patient with primary hyperparathyroidism

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Introduction
Despite the coexistence of secondary hyperparathyroidism with anaemia, hematological manifestations of primary hyperparathyroidism (PHPT) are rare.
Case report
A 67-year-old Caucasian female was admitted to the Department of Internal Medicine due to normocytic anemia and hypercalcemia, diagnosed on occasion of muscle weakness and fatigability for 3 months.
Her medication included: alendronate 70 mg/week, alphacalcidol 1 μg/day, valsartan 160 mg, hydrochlorothiazide 12.5 mg/day, oxcarbazepine 900 mg/day and simvastatin 40 mg/day. Her family history was negative for diseases affecting bone metabolism. No remarkable signs were revealed from clinical examination.
Initial laboratory assessment showed: hematocrit (Ht): 28.6%, hemoglobin (Hb): 9.3 g/dl, white cell count: 6760/μl, platelets: 281 000/μl, urea: 13 mg/dl, creatinine: 1.35 mg/dl, estimated glomerular filtration rate (eGFR): 44 ml/min per 1.73 m^2, serum total calcium (Ca): 14.3 mg/dl (normal: 8.8–10.6), serum phosphorus (P): 4.9 mg/dl (normal: 2.5–4.5), parathyroid hormone (PTH): 350 pg/ml (normal: 10–53), 25-hydroxyvitamin-D: 7.9 ng/ml (normal: >30 ng/ml), 24 h urinary Ca: 300 mg/24 h (normal: 0–250). Neck ultrasound revealed a hypoechoic lesion 21×5.4 mm suggestive of adenoma of the right lower parathyroid gland, confirmed by Tc99m-sestamibi scan.
Regarding anaemia, comprehensive laboratory, endoscopic and imaging investigation was negative. Bone marrow biopsy and myelogramm showed normal cellularity without fibrosis.
The patient was initially managed with fluid resuscitation, i.v. furosemide and cinacalcet, after alphacalcidol and hydrochlorothiazide discontinuation, which resulted in gradual restoration of renal function and improvement of Ca (10.9 mg/dl). The patient underwent a successful parathyroidectomy, with postoperative PTH: 31.8 pg/ml, Ca: 9.8 mg/dl and P: 3.4 mg/dl.
Surprisingly, Ht and Hb returned to normal postoperatively. In particular, 3 months later, Ht was 34.5% and Hb: 11.5 g/dl, while after 12 months, Ht: 39.8% and Hb: 13.4 g/dl.
Conclusions
Successful parathyroidectomy resulted in resolution of anaemia. The etiology of anaemia is not fully elucidated. Proposed mechanisms are the development of marrow fibrosis and the inhibitory effect of PTH on erythropoiesis.
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P108

A case of 23 years old woman with primary hyperparathyroidism presenting with pathological fracture and brown tumors detected by scintigraphy

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Introduction
Primary hyperparathyroidism (PHPT) occurs a peak incidence between ages 50 and 60 and the classical bone disease of PHPT or pathological fractures due to PHPT is rarely seen today. Furthermore brown tumor detected with Tc-99m MIBI scintigraphy exists in literature infrequently.
Case
A 23 years old woman presented with left arm pain admitted in Department of Orthopaedics for pathological fracture in left humerus. As a result of investigations hypercalcemia and cystic lesions in diaphysis of left humerus and many other bone localization found, then referred to our section.
Initial laboratory profile, serum calcium: 15.2 mg/dl (8.6–10.2), serum phosphorus: 2.5 mg/dl (2.6–4.5), total alkaline phosphatase: 722 U/l (35–105), 24-h urine calcium: 572 mg/day (100–321), PTH (IRMA): 3308 ng/ml (15–65), 25(OH)vitamin D: 8.98 ng/ml (25–80). Rest blood results revealed normal. Also there was no abnormality at plasma calcitonin, metanephrine, normetanephrine, anterior pituitary hormones and basal cortisol levels.
Neck ultrasound showed an encapsulated, homogeneous, hypoechoic solid mass lesion at inferior pole of right lob of tyhroid gland, largest measures $2\times2\times1.5$ cm in dimension, contains central and peripheral vascularity.
Tc-99m MIBI scintigraphy was performed and a foci of radiotracer accumulation was seen caudal to the right lobe of thyroid gland, also found accumulations on distal third of the left clavicle and the localizations of both sternoclavicular joints consistent with brown tumor.
Evaluation of bone mineral density; the lowest *Z*-score value in localization lumbar region and total value of the femur were recorded as (-3.2) and (-1.9), respectively.
After seum calcium values lowered the patient was operated and localized parathyroid gland was excised. The pathological evaluation revealed parathyroid adenoma.
Conclusion
PHPT also occurs at a young age therefore familial hyperparathyroidism syndromes such as MEN should be investigated in these cases. Nowadays despite most patients with PHPT do not have the classic radiologic bone disease, the skeleton lesions can be seen and even lead to pathological fractures. Furthermore cystic lesions in bones can be evaluated inaccuracy primary bone tumor.
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P109

A patient osteogenesis imperfecta with osteoporosis

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Introduction
Osteogenesis imperfecta (OI) is a congenital, generalized connective tissue disorder characterized by severe osteoporosis and bone fragility. OI is most commonly caused by mutations in genes encoding the α-1 and α-2 chains of type I collagen or proteins involved in posttranslational modification of type I collagen. Although, no controlled studies are done, bisphosphonates are used for the treatment of OI. I.v. bisphosphonates are reported to be more effective for pain conrol than oral bisphosphonates. Here, we report the results of a patient with OI and osteoporosis who received cyclic i.v. pamidronate.
Case report
A 31 years old man with OI and osteoporosis admitted with generalized bone pain. He had been using alendronate 70 mg/week p.o. for 2 years with no improvement in bone mineral density (BMD) and pain. I.v. cyclic pamidronate was started at a dose of 60 mg/day for 3 days every 4 months. BMD was assessed at baseline, at 6 and 12 months of treatment. Short form-36 (SF-36) questionnaire was used to evaluate his quality of life at baseline and at 12 months. His vertabral BMD *T*-score was −4.2 and femur neck *T*-score was −2.0 at baseline. At 6th month, his vertabral BMD *T*-score was −3.6 and femur neck *T*-score was −1.6. At 12th month, his vertabral BMD *T*-score was −3.6 and femur neck *T*-score was −1.4. A significant improvement was observed in his SF-36 scores at 12th month. No adverse effects were observed due to treatment.
Conclusions
OI is a rare disease and no treatment protocol has been shown to be superior to others. I.v. bisphosphonates may be better for pain control than oral bisphosphonates. This case shows the improvement of BMD and quality of life after 1 year treatment with i.v. pamidronate, which not achived with oral alendronate.

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P110

Fear of illness and quality of life in Greek postmenopausal women with osteoporosis

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Introduction
Fear of illness and quality of life have been proven to be affected in women with osteoporosis. Osteoporosis causes an increase in morbidity and mortality but the direct ways by which it affects fear of illness and quality of life are not yet known. The present study aims to assess the fear of illness and quality of life (QoL) as well as their correlation with bone density and age in a Greek rural population of postmenopausal women with osteoporosis.
Methods
Sixty postmenopausal women, aged 50–85 years were studied. Bone mineral density (BMD) was estimated in all the women with the use of Achilles InSight – Bone Ultrasonometer. Every woman who had *T*-score <-2.5 completed a questionnaire for assessing health related quality of life in osteoporosis (ECOS-16). Higher scores in ECOS-16 indicate lower quality of life.
Results
Twenty-five women with a mean age 70 ± 7 years had a *T*-score <-2.5. Older women had greater fear of their illness ($P<0.05$) with age being the only variable correlated with fear of illness ($P<0.05$). Women with lower *T*-scores had reduced quality of life ($P<0.05$).
Conclusion
The grade of osteoporosis can significantly influence the quality of life whereas fear of illness is also influenced in older postmenopausal women. Local health services should focus on prevention and treatment of osteoporosis as well as on the support and improvement of quality of life of women living with osteoporosis.

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Calcium and Vitamin D metabolism

P111

The relationship between low maternal serum 25-hydroxyvitamin D level and gestational diabetes mellitus according to the severity of 25-hydroxyvitamin D deficiency

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Introduction
The results of publications investigating the relationship between low maternal serum 25-hydroxyvitamin D (25OHD) level and gestational diabetes mellitus (GDM) are controversial and none of these publications have investigated this relationship according to the severity of 25OHD deficiency. Therefore, this study was conducted to assess the relationship between low maternal serum 25OHD and GDM according to the severity of 25OHD deficiency.
Methods
We analysed serum 25OHD levels in 234 women with GDM and 168 controls. To define deficiency status, maternal serum levels of 25OHD were further classified into four groups (<12.5 nmol/l as severely deficient, 12.5–24.9 nmol/l as deficient, 25–49.9 nmol/l as insufficient and ≥ 50 nmol/l as sufficient, respectively).
Results
Women with GDM had significantly lower 25OHD levels compared to controls (30.8 ± 16.3 vs 36.0 ± 16.2 nmol/l, $P=0.002$). However, when subgroups of 25OHD were analysed, GDM was significantly more common only in women with severely deficient 25OHD level. After adjusting for covariates including maternal age, previous history of GDM, history of type 2 DM in first degree relatives and pre-pregnancy BMI, only severely deficient 25OHD levels were independently associated with an increased relative risk of GDM (OR = 3.95, 95% CI, 1.68–9.25, $P=0.002$). The odds ratios of GDM in women with insufficient and deficient 25OHD levels were not statististically significant (OR = 1.46, 95% CI, 1.27–2.74, $P=0.23$, OR = 1.64, 95% CI, 1.26–3.43, $P=0.18$, respectively). PTH concentrations were also significantly higher in women with GDM compared to controls (45.3 ± 26.2 vs 38.7 ± 27.6 pg/ml, $P=0.016$).
Conclusions
Results from this study provide novel data indicating that only severely deficient maternal serum 25OHD level is significantly associated with an elevated relative risk of GDM, even after adjusting for established risk factors of GDM. Large-scale, well-designed and multi-center studies are required to further evaluate this relationship.

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P112

Effects of vitamin D fortified bread on muscle strength

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Background
Vitamin D deficiency is common in elderly nursing home residents. It has been associated with low bone mineral density, muscle weakness, increased body sway and falls. Evidence indicates that supplementation of vitamin D in these individuals helps to improve locomotor function and general health.
Patients and methods
We evaluated, in a 12 months interval, the effects of vitamin D supplementation on grip strength in 45 nursing home residents (28 women and 17 men, aged 58–89 years) who consumed daily one bun fortified with 125 μg vitamin D_3 (25(OH)D) and 320 mg elemental calcium, in order to achieve optimal blood levels of 25(OH)D >75 nmol/l. Grip strength was measured at baseline and 12 months after vitamin D supplementation, using a specific dynamometer (Baseline Squeeze Bulb Dynamometer, USA). For each hand, the highest of three attempts was noted as maximal grip strength. The average of maximum values for the left and right hands was considered as the measure of the participant's muscle strength.
Results
Muscle strength positive correlated with locomotion functions score ($P=0.034$) and levels of vitamin D ($P=0.046$), but significant improvement of grip strength could not be proven although optimal levels of vitamin D were maintained for long term. Although optimal levels of 25(OH)D were associated with an amelioration of locomotor function, grip strength improvement was not statistically significant ($P=0.570$).

Conclusions
Vitamin D deficiency is only one of the conditions that affect muscle function in elderly people. Our results showed a good correlation between muscle strength and 24(OH)D_3 levels, but sustained normalisation of vitamin D levels is not sufficient for significant improvement of muscle function. Further control randomized studies may be necessary for a better understanding of the effects of long term vitamin D supplementation on the health outcomes in the elderly.

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P113

Evaluation of two routinely used 25OHD assays and serum variables in patients on dialyses

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The total 25-hydoxy-vitamin-D (t25OHD) level can be assessed by various methods and reflects vitamin D intake. Results are influenced by the serum variables affected by dialyses.
Aims
To examine t25OHD and bioavailable vitamin D (b25OHD) by two methods in patients on peritoneal-(PD) and hemodialysis (HD).
Methods
We studied 37 HD (64 ± 15 years) and 36 PD (63 ± 18 years) patients. The t25OHD was assessed by chemiluminescence immunoassay (LIA) and electrochemiluminescence protein binding assay (PBA). Levels of PTH-biointact (PTHbi) by immunometric assay, vitamin D binding protein (DBP) by turbidimetry and albumin by colorimetry were measured. The b25OHD values were calculated.
Results
t25OHD values below the analytical sensitivity occurred more frequently by PBA (29%) than by LIA (1.4%). Values of t25OHD (LIA: 23 ± 8 vs 37 ± 15; PBA: 11 ± 6 vs 37 ± 19 nmol/l) and b25OH (LIA: 2.1 ± 1.5 vs 4.7 ± 5.1; PBA: 1.0 ± 0.7 vs 4.8 ± 5.8 nmol/l) were lower in PD, but PTHbi levels were lower in HD (150 ± 92 pg/ml) than in PD (287 ± 152 pg/ml; $P<0.001$). Albumin levels were lower in PD (38 ± 5 vs 41 ± 4 g/l; $P<0.01$), but DBP levels were higher in PD than in HD (339 ± 102 vs 291 ± 112 mg/l; $P<0.05$). Positive correlations were observed with both methods between t25OHD and albumin levels in PD (PBA: $r=0.36$; $P<0.05$; LIA: $r=0.48$; $P<0.01$). The correlations of t25OHD levels assessed by LIA and BPA were different (HD: $r=0.89$; $P<0.001$; PD: $r=0.47$, $P<0.01$), but correlations of b25OHD values were similar (HD: $r=0.85$; PD: $r=0.83$, $P<0.001$) in both groups. Negative correlations were observed between PTHbi and t25OHD levels (PBA: $r=-39$, LIA: $r=-0.42$; $P<0.05$) in HD only, but between PTHbi and b25OHD were similar in both (LIA: PD $r=-0.40$, HD $r=-0.54$, $P<0.01$; PBA: PD $r=-0.49$, HD $r=-0.44$).
Conclusions
Assessment of vitamin D supply by LIA and PBA is influenced by lower albumin levels especially in PD. Estimation of b25OHD is more suitable in PD, but t25OHD is reliable by both methods in HD.

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P114

Vitamin D supply in healthy women with different reproductive stages: is there any relationship with DBP levels?

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The total 25-hydoxy-vitamin-D (t-25OHD) level reflects the supply of vitamin D, but is also influenced by the levels of 25OHD binding proteins (DBP). We aimed studying t-25OHD and bioavailable 25OHD (bio-25OHD) levels in healthy females with different reproductive ages.
Methods
t-25OHD, bio-intact parathormone (PTHi-1–84) (ECLIA, cobas e411, Roche), DBP (turbidimetry, Dako), calcium and albumin (Alb) levels were measured by routine methods (Modular, Roche). Bio-25OHD and albumin – corrected Ca (AlbCa) were calculated in 126 healthy young women (age: 35.3 ± 15.9 years) of whom 41 took oral contraceptives (OC); 45 were without pills (C1) and 40 were pregnant (PRG). An older control group of 21 postmenopausal women was also included (C2: age: 68.5 ± 14.5 years).
Results
The highest levels of DBG were found in the PRG and OC groups. Based on the t-25OHD levels, only 12% of women in the OC, but 36% of women in the C1 group had suboptimal (<75 nmol/l) vitamin D supply. Bio25-OHD values reflecting suboptimal (<6.4 nmol/l) vitamin supply occurred most frequently (51%) in the OC and least frequently in the C1 group (15%). Levels of t-25OHD were highest ($P<0.001$) in the OC (100 (88 117) nmol/l) and the lowest ($P<0.001$) in PRG group (41 (28 57) nmol/l). However, bio-25OHD levels were lower in the OC (6.3 (5.6 8.1) nmol/l) than in the C1 group (8.1 (6.6 9.7) nmol/l $P=0.06$). Associations were observed between t-25OHD and PTH-1–84 levels in all groups (C1: $\beta=-0.29$ $P<0.05$; C2: $\beta=-0.62$ $P<0.01$; OC: $\beta=-0.36$ $P<0.05$) except the PRG group. There was no association between t-25OHD and CaAlb levels, while a correlation was detected between bio-25OHD vitamin and CaAlb levels ($\beta=0.53$ $P<0.05$) in the C2 group only.
Conclusions
t-25OHD and DBP levels may dysproportionally change. The estimates of vitamin D supply are influenced by the 25OHD fraction assessed especially in case of estrogen excess.

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P115

Cinacalcet in patients with primary hyperparathyroidism (PHPT): comparison between sporadic and MEN1 PHPT

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Introduction
PHPT is a common endocrine disease characterized by hypercalcemia and different degree of osteoporosis and nephrolithiasis. PHPT arises in the context of an inherited disease in 5% of cases. Multiple endocrine neoplasia type 1 (MEN1) is the commonest cause of inherited PHPT. The main therapeutic approach for PHPT is surgery, however many patients refuse or have contraindications for surgery while others, in particular those with MEN1 PHPT, have persistent/relapsing disease after surgery. Cinacalcet is a calciomimetic agent which has been demonstrated to be effective in the control of PHPT.
Aim
The aim of this study was to evaluate and compare the effectiveness of cinacalcet in patients with sporadic and MEN1 PHPT.
Patients and methods
Fifty patients have been enrolled: 25 of them had sporadic PHPT (7 M, 18 F, mean age 67 years) and 25 MEN1 PHPT (9 M, 16 F, mean age 41 years). Serum concentrations of PTH, calcium and phosphorus were evaluated before and 6 and 12 months after starting cinacalcet.

Results
Serum calcium and PTH concentrations significantly decreased in both groups ($P<0.01$). There were not significant differences between sporadic and MEN1 PHPT in the rate of calcium and PTH concentration decrease after 6 and 12 months of therapy. At 12 month follow-up, the dose of cinacalcet required to normalize calcemia in sporadic PHPT was 30 mg a day in 15 patients and 60 mg in 10 other patients, while in MEN1 PHPT the dose of cinacalcet was 30 mg in 8 patients, 60 mg in 11, 90 mg in 6 other patients.
Conclusions
Cinacalcet is equally effective in normalizing hypercalcemia in sporadic and MEN1 PHPT. However, MEN1-related PHPT required a higher dose of cinacalcet than sporadic PHPT to normalize serum calcium. This contrasts with preliminary published reports and might be explained by the fact that in MEN1 PHPT all parathyroid tissue is affected and hyperfunctioning.

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P116

Efficacy of vitamin D on COPD exacerbation: a double blind randomized placebo-controlled clinical trial

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Background
Exacerbations are an important feature and outcome measure in chronic obstructive pulmonary disease (COPD). The objective of this study was to investigate the effect of supplementation of standard treatment (inhaled long-acting β2 agonists, anticholinergics and corticosteroids) with vitamin D on pulmonary function tests in patients with COPD exacerbation.
Methods
Patients referred to hospital were divided into three groups receiving 0.25 μg calcitriol daily ($n=45$), 50 000 IU daily of vitamin D ($n=45$) and placebo ($n=45$). Spirometry and clinical assessment were carried out at the beginning and after 1 week treatment.
Results
There was a similar degree of lung function and clinical improvement in all three groups ($P>0.05$). C-reactive protein level did not significantly different among three groups of treatment at 1 week after treatment.
Conclusions
Our findings showed that 1 week supplementation of treatment with 50 000 IU daily of vitamin D_3 or 0.25 μg calcitriol daily did not provide any additional clinical benefit.

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P117

Lower CD4 lymphocyte counts in HIV/HCV co-infected patients with liver fibrosis on long term HAART and low vitamin D level

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Introduction
Vitamin D insufficiency (D-INSUFF) has been associated with impaired immune response in human inmunodeficiency virus (HIV) patients and a worse prognosis in patients with liver fibrosis. Nevertheless, few studies have explored the influence of D-INSUFF in immunological response and liver parameters in HIV and hepatitis C virus co-infected patients (HIV/HCV) on long term highly active antiretroviral therapy (HAART) with successful immune and virological response. In this cross-sectional study, first we determined the prevalence of D-INSUFF in a cohort of HIV/HCV outpatients with liver fibrosis and second, we assessed whether the existence of D-INSUFF involves the appearance of relevant immunological data and/or particular clinical aspects related to liver disease.
Methods/design
Thirty-six consecutive HIV/HCV co-infected men (mean age: 48 ± 5.1 years) with liver fibrosis on long term HAART were included in this study. D-INSUFF was defined as 25OH-D levels ≤30 ng/ml. Liver fibrosis was defined as the presence of a liver stiffness ≥9 kPa measured by FibroScan. Age, data related to HIV and HCV infection, anthropometric, nutritional and metabolic parameters were recorded. Child-Pugh and Model for End-Stage Liver Disease (MELD) were used for assessing the severity of chronic liver disease.
Results
The mean serum 25(OH)-D concentration was 29.1 ± 11.5 ng/ml, with a prevalence of D-INSUFF of 61.1% (mean level: 21.7 ± 4.3 ng/ml). D-INSUFF patients as compared to patients who had normal levels of 25OH-D significantly had lower CD4 lymphocyte count ($462.2\pm170.5/mm^3$ vs $745.9\pm107.3/mm^3$; $P=0.02$) and lower serum albumin levels (4.4 ± 0.2 vs 4.8 ± 0.3 g/dl; $P=0.05$). Serum PTHi, calcium and phosphorus levels, liver stiffness value, Child-Pugh and MELD scores were not significantly different among D-INSUFF patients or those with normal 25OH-D level.
Conclusions
Although the level of CD4 lymphocytes is adequate in both groups, the immunological response to HAART is less effective in HIV/HVC co-infected patients with vitamin D insufficiency. Strategies to supplement vitamin D in these patients may help to improve immune status.

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P118

Replenishment of reduced vitamin D levels: how much vitamin D is necessary?

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Reduced vitamin D levels are a prevalent clinical finding that is clearly associated with increased morbidity and mortality. There is considerable controversy, however, as to how much vitamin D is required for the effective vitamin D replenishment in patients with reduced vitamin D levels.
Therefore, we decided to evaluate the effectiveness of 1000 to 3000 U qd of vitamin D and of vitamin D receptor agonists (VDRA) in 161 patients with vitamin D levels ≤25 nmol/l (group A) and 104 patients with vitamin D levels of 26–50 nmol/l (group B). 209 of the patients were women and 56 men (mean age, 47 ± 1 years (S.E.M.)). All patients had normal kidney function but GGT was higher in group A (31 ± 3 vs 21 ± 1 U/l, $P<0.05$). Reduced vitamin D levels were associated with lactose intolerance (40% of patients), fructose malabsorption (24%), pancreatic insufficiency (12%) and coeliac disease and/or IgA deficiency (11%). Calcium and phosphate in serum, PTH and 25-OH-vitamin D levels or 1,25-OH-vitamin D levels, respectively, were measured by routine methods.
19% of the untreated patients had hypocalcaemia and 28% had secondary hyperparathyroidism (SHPT). 3000 U of vitamin D resulted in vitamin D levels >50 nmol/l in 82% of the patients in group B but only in 54% of the patients in group A ($P<0.01$). Accordingly, SHPT was controlled in 94% of the patients in group B but only 85% of the patients in group A. VDRA controlled SHPT in 98% of patients in group A ($P<0.05$). In both groups, 1000 and 2000 U of vitamin D were less effective in normalizing vitamin D or calcium levels and in correcting SHPT ($P<0.001$). There was no case of hypercalcaemia or increased vitamin D or VDRA levels in the course of the study.
In patients with reduced vitamin D levels, vitamin D doses up to 3000 U/day or VDRA, respectively, are required to safely and effectively normalize vitamin D levels, to control prevalent hypocalcaemia and SHPT and to, presumably, prevent its sequelae.

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P119

Changes in anxiety and depression symptoms in patients with chronic fatigue syndrome treated with vitamin D

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Introduction
Vitamin D is involved not only in the regulation of calcium–phosphorus metabolism; it also plays an important role in maintaining the immune system,

muscle strength and neuromuscular conduction. Lack of vitamin D is assumed as one of the mechanisms of autoimmune and infectious diseases. Chronic fatigue syndrome (CFS) – is a syndrome manifesting with a severe chronic physical, mental weakness, psychological disturbances such as anxiety and depression, the development of which is associated with impaired immunity, chronic infectious diseases and brain dysfunction. There are published data on the high prevalence of low levels of vitamin D in the Finnish cohort of patients with CFS.
Aim
To assess the prevalence of vitamin D insufficiency, changers in anxiety and depression scores after treatment with 150 000 IU of cholecalciferol in patients with and without CFS.
Materials and methods
The study included 81 women aged 40–60 years (median age – 53 ± 4 years), 29 of which were diagnosed with the CFS using CDC criteria (1994). Vitamin D levels were evaluated during the period from late June till September by LIASON 25OH total vitamin D assay (DiaSorin, Inc.). Beck depression and Zung anxiety scales we completed by patients before and after treatment.
Results
The prevalence of vitamin D deficiency, defined as a level <20 ng/ml was 54% in patients with CFS and 74% in the control group ($P>0.05$), the optimal level of vitamin D (30 ng/ml) was observed in 10 and 14% of patients, respectively ($P>0.05$). Medical therapy with cholecalciferol (25 000 IU a week orally during 6 weeks) resulted in decrease in depression scores from 35 to 13 in CFS+ group ($P=0.00004$) and insignificantly from 11 to 9.5 in CFS− group ($P>0.05$); decrease in anxiety scores from 38 to 35 in CFS+ group ($P=0.00003$) and from 28 to 22 in CFS− ($P=0.0000000007$); decrease in number of minor diagnostic criteria symptoms in patients with CFS from 5.4 to 4.9 ($P=0.04$). The studied parameters did not correlate with levels of vitamin D before and after treatment and with the net change in levels.
Conclusions
There is a high prevalence of vitamin D deficiency in perimenopausal women during period of sufficient insolation irrespective of CFS presence. Vitamin D treatment seems to improve psychological functioning but the mechanisms might not be direct.

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The effects of vitamin D therapy on thyroid functions, thyroid autoantibodies, TNF-α, IL6 and IL1b in patients with autoimmune thyroiditis

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Vitamin D has immunomodulatory features and its deficiency is associated with the development of autoimmune diseases. The relation between vitamin D deficiency and autoimmune thyroiditis has not been investigated much. In the present study, our aim was to study the relation between vitamin D therapy and autoimmune thyroiditis.
Fifty-four patients having newly diagnosed Hashimoto thyroiditis (HT) and vitamin D deficiency but requiring no thyroid hormone replacement therapy at the time of enrolment to the study were included in the study. Once every 3 months, the patients received i.m. cholecalciferol injection (300.000 IU). During diagnosis and at the end of vitamin D therapy, thyroid functions, thyroid antibodies, $25(OH)D_3$, PTH, Ca, P and ALP levels were in each patient while TNF-α, IL6 and IL1b levels were measured in only 43 patients.
When pre-therapy and post-therapy levels of FT_4, TSH, antiTPO, antiTG, PTH and ALP were compared, there was a significant difference ($P<0.05$). While there was a significant increase in FT_4 levels after the therapy, the decrease in TSH, antiTPO, antiTG, PTH and ALP levels was significant. There was no significant difference in terms of FT3, Ca, P, TNF-α, IL6 and IL1b. The was no correlation between pre-therapy and post-therapy vitamin D levels and FT_3, FT_4, TSH, antiTPO, antiTG, PTH, Ca, P, ALP, TNF-α, IL6 and IL1b.
An increase in FT_4 level and a decrease in TSH, antiTPO and antiTG levels after a 3 months vitamin D therapy in patients having HT and vitamin D deficiency but requiring no LT_4 therapy suggest that HT may have a positive effect on thyroid antigenicity and function. Autoimmune thyroiditis frequently progresses into subclinical hypothyroidism and overt hypothyroidism after euthyroid phase. Such a progress can be slowed down or prevented by vitamin D therapy in HT patients having vitamin D deficiency.

Key words
Autoimmune thyroid diseases, Hashimoto's thyroiditis, Vitamin D deficiency.
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P121

Genetic analysis of *AIP* genes in familial primary hyperparathyroidism

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Primary hyperparathyroidism (PHPT) is usually a sporadic disorder, but in $<10\%$ of cases occurs as part of hereditary syndromes, including multiple endocrine neoplasia types 1 and 2A (MEN1 and MEN2A), hyperparathyroidism–jaw tumor syndrome (HPT–JT) and familial isolated hyperparathyroidism (FIHP).
MEN1 is an autosomal dominant disorder characterized by tumours in multiple endocrine glands, most commonly parathyroid, enteropancreatic and anterior pituitary glands. To date *MEN1* gene germline mutations have been identified in 70–80% of MEN1 kindreds. FIHP has a heterogeneous molecular ethiology, since germline mutations of *MEN1*, *HRPT2* and *CASR* genes have been reported. Germline mutations of the aryl hydrocabon receptor interacting protein (*AIP*) gene, responsible for 15–25% of familial isolated pituitary adenoma (FIPA) syndrome, have been recently reported in a MEN1 case.
The aim of this study was to perform a genetic screening of *AIP* gene in 22 MEN1 and 14 FIHP kindreds. All MEN1 kindreds were negative for *MEN1* gene mutations and all FIHP families were negative for *MEN1, HRPT2, CASR* mutations at genetic testing.
Genomic DNA from index cases was analyzed by PCR amplification of the entire coding region and splice sites and direct sequencing by a 16-capillaries automatic sequencer.
Two germline mutations in exon 1 of *AIP* gene were detected in two MEN1 probands, namely Arg9Gln (c.26G>A) and Arg16His (c.47G>A). Both mutations have already been reported, Arg9Gln in an acromegalic patient, and Arg16His in several FIPA families and patients with apparent sporadic pituitary adenoma. R9Q variant has been described to cause a significant increase in proliferation in cell cultures, while the pathogenetic nature of R16H is still under investigation since it has been identified in few healthy subjects and in some families seems not to segregate with the disease.
Our results suggest that germline *AIP* mutations may be involved, although rarely, in parathyroid tumorigenesis.

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P122

A986S or the R990G polymorphism in CASR does not explain hypercalciuria and low normal serum calcium

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The calcium receptor (CASR) serves as one of the main regulators of the calcium homeostasis. CASR is expressed in among other tissues parathyroid chief cells and kidney tubule cells. It has been hypothesized that *CASR* gene variations are responsible for low circulating calcium levels together with hypercalciuria and thereby increased risk of kidney stones. The *CASR* gene polymorphism A986S has been shown associated to elevated serum calcium levels *in vivo*. On the opposite R990G has been shown associated to low serum calcium levels.
Aim
Are the polymorphisms A986S and R990G overrepresented in hypercalciuria patients with low normal serum calcium levels.
Method
The *CASR* gene was sequenced in 109 Italian out-patient Caucasians suffering hypercalciuria (calcium excretion >4 mg calcium/day). The patients had low normal serum calcium values and serum PTH within the normal range. All had normal kidney function.
Results
Sixty-eight of 109 patients (62%) showed at least one single-nucleotide polymorphism (SNP). Up to 4 SNP's were found in each patient, all well characterised. Of these 37 patients (28%) presented A986S, 12 patients (13%) the R990G polymorphism.
Discussion
The observed A986S and R990G distribution corresponds to the frequency and genotype shown in the ancestry population of Northern and Western European.
Conclusion
A986S or the R990G polymorphism does not explain hypercalciuria and low normal serum calcium in this cohort.

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P123

Early measure of postoperative iPTH and corrected calcium as predictors of future hypoparathyroidism: which, when and why?

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Introduction
Intraoperative parathyroid hormone assay (ioPTH) has been validated as a useful tool for predicting postoperative hypocalcaemia (hypoCa) after thyroid surgery and has been proposed as a guide to early discharge from hospital. Its value to predict the risk of future hypoparathyroidism (hypoPT) has not been analyzed in detail. We evaluate this role in our recent surgical series.
Description of methods/design
Patients with total thyroidectomy from 2005 to 2011, evaluated more than 1 year after surgery. Permanent hypoPT (PhypoPT) was defined by (iPTH) <15 pg/ml without treatment 1 year after surgery. Cases with spontaneous recovering of parathyroid function after a period of (iPTH) <15 were named as transient hypoPT (ThypoPT). We analyze the correlation between (iPTH) measured 24 h after surgery (iPTH24 h) and future parathyroid function. We also analyze the correlation between (iPTH) measured immediately (1–3 h) after surgery (iPTH1–3 h), corrected calcium monitored 6 h postoperatively (Ca6 h) and subsequent parathyroid status.
Results
502 patients had (iPTH24 h), 305 of them also had (iPTH1–3 h). Of 125 with (iPTH24 h) <3 pg/ml, 40 presented PhypoT. The remainders were transient forms, 56% corrected in the 1st month after surgery. Mean (S.D.) (iPTH24 h) in patients without hypoPT was 39.13 (23.45) vs 3.54 (3.54) in patients with hypoPT ($P<0.0001$). Mean (S.D.) (iPTH1–3 h) was 34.88 (28.02) and 3.10 (7.9) pg/ml ($P<0.0001$) respectively. Median (iPTH24 h) and (iPTH1–3 h) in patients without hypoPT were 33.5 and 30 pg/ml ($P<0.001$). Postoperative hypoCa, defined as corrected plasmatic calcium <7.5 mg/dl, presented in 183/647 patients including 66.3% of patients with (iPTH24 h) <10 pg/ml, vs 10.9% of them with (iPTH24 h) ≥ 10 pg/ml (OR: 3.48 (CI: 2.6–4.64)). The OR for postoperative hypoCa with (iPTH1–3 h) <10 pg/ml was 3.89 (CI: 2.48–6.10). (iPTH24 h) >15 pg/ml was present in 5% of patients with hypoPTH and (iPTH1–3 h) >15 pg/ml in 9.5%, all ThypoPT. Mean (Ca6 h) was significantly lower in patients who suffered hypoPT (8.63 vs 8.22; $P<0.001$).
Conclusion
Our study confirms the utility of perioperative (PTH) as predictor of postoperative hypoCa and future hypoPT, but we have not found an exact level or timing for its measurement.

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Serum sclerostin and Dkk1 in patients with parathyroid disorders

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The 'canonical' Wnt/β-catenin pathway plays an important role in the development and patterning of bone. Dkk1 (Dickkopf1) and sclerostin are competitive soluble inhibitors of this pathway. Serum sclerostin is decreased in patients with primary hyperparathyroidism (PHPT) compared to the healthy subjects and hypoparathyroid (HypoPT) patients. No data are currently available on Dkk1 serum level in PHPT.
We evaluated serum Dkk1 and sclerostin levels in 42 (10 males and 32 females) patients with sporadic PHPT, 16 (9 males and 7 females) patients with HypoPT and 36 (10 males and 26 females) healthy controls.
Serum sclerostin and Dkk1 were measured using an ELISA assay (Biomedica Medizinprodukte GmBH & Co KG., Germany). All measurements were performed in a single assay. Bone mineral density at lumbar spine, hip and third distal non dominant forearm was measured in patients with PHPT by DXA (QDR-Hologic).
Serum sclerostin concentration in PHPT patients (15.8 ± 7.0 pmol/l) was lower than in controls (20.4 ± 7.9 pmol/l, $P=0.0052$), whereas no difference was found between PHPT and HypoPT patients (18.4 ± 8.1 pmol/l, $P=0.198$) and between HypoPT patients and controls ($P=0.382$). Serum Dkk1 concentration was lower in PHPT (6.2 ± 2.6 pmol/l, $P<0.0001$) and HypoPT patients (6.3 ± 2.3 pmol/l, $P=0.0026$) compared to healthy subjects (9.2 ± 3.6 pmol/l), whereas no difference was found between PHPT and HypoPT patients ($P=0.940$). In PHPT patients, there was a negative correlation between serum PTH and sclerostin ($r=0.342$, $P=0.025$), and no correlation between PTH and Dkk1 level. No correlation was found between sclerostin, Dkk1 and bone turnover markers, bone density.
In conclusion, serum levels of sclerostin and Dkk1 are decreased in patients with PHPT compared to controls; no definitive conclusion can be reached in patients with HypoPT because of the limited number of patients.

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P125

An observational study reveals that neonatal vitamin D is primarily determined by maternal contributions: implications of a new assay on the roles of vitamin D forms

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Introduction
Vitamin D concentrations during pregnancy are measured to diagnose states of insufficiency or deficiency. The aim of this study is to apply accurate assays of vitamin D forms (single hydroxylated ($25(OH)D_2$, $25(OH)D_3$), double-hydroxylated ($1\alpha,25(OH)_2D_2$, $1\alpha,25(OH)_2D_3$), epimers (3-epi-$25(OH)D_2$, 3-epi-$25(OH)D_3$)) in mothers (serum) and neonates (umbilical cord) i) to explore maternal and neonatal vitamin D biodynamics and ii) to identify maternal predictors of neonatal vitamin D concentrations.

Methods
All vitamin D forms were quantified in 60 mother–neonate paired samples by a novel mass spectrometry (LC–MS/MS) assay. Maternal characteristics (age, ultraviolet B exposure, dietary intake, calcium, phosphorus and parathyroid hormone) were recorded. Hierarchical linear regression was used to predict neonatal 25(OH)D concentrations.
Results
Mothers had similar concentrations of $25(OH)D_2$ and $25(OH)D_3$ forms compared to neonates (17.9 ± 13.2 vs 15.9 ± 13.6 ng/ml, $P=0.289$) with a ratio of 1:3. The epimer concentrations were similar in mothers and neonates (4.8 ± 7.8 vs 4.5 ± 4.7 ng/ml, $P=0.556$). Neonatal $25(OH)D_2$ was best predicted by maternal characteristics, whereas $25(OH)D_3$ was strongly associated to maternal vitamin D forms ($R^2=0.253$ vs 0.076 and $R^2=0.109$ vs 0.478, respectively). Maternal characteristics explained 12.2% of the neonatal 25(OH)D, maternal 25(OH)D concentrations explained 32.1%, while epimers contributed an additional 11.9%.
Conclusions
By applying a novel highly specific vitamin D assay, the present study is the first to quantify 3-epi-25(OH)D concentrations in mother–newborn pairs. Maternal characteristics and active forms of vitamin D, along with their epimers explain 56% of neonate vitamin D_3 concentrations. The roles of active and epimer forms in the maternal–neonatal vitamin D relationship warrant further investigation.

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Vitamin D and sarcopenia in HIV-infected patients

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Introduction
Sarcopenia, an age-associated loss of skeletal muscle mass and function, has been related to higher mortality in general population as well as in HIV-infected patients. In elderly, sarcopenia is a marker of frailty. The aim of our study was to describe the prevalence of sarcopenia and related factors in HIV-infected population.
Methods
Skeletal muscle mass (SMM), total fat mass and body fat distribution were measured by DXA scan. Muscle mass index (MMI) was calculated (lower limb SMM/height^2). Sarcopenia was defined as an MMI <2 SD from observed in general population (7.26 kg/m^2 males, 5.5 females). Body fat distribution was determined by body fat index (trunk fat mass/limb fat mass). Plasma levels of 25-OH-vitamin D (VD) were measured by immunoassay.
Results
321 HIV-infected patients were studied. 85% were male, median age was 38 years (IQR 32–45), BMI was 23.8 (IQR 22–26) kg/m^2; 45% naive and 54% on antiretroviral therapy (ART). Previous AIDS diagnosis was present in 20%; median CD4 lymphocyte count was 454/μl (RIQ 307–615), HIV-1 viral load was undetectable (<50 cop/ml) in 84.3% of those on treatment. Hypovitaminosis D (<30 ng/ml) was present in 77%, being severe (<10 ng/ml) in 17%. The prevalence of sarcopenia was 23.5%. A low BMI, low waist circumference and less central body fat distribution were significantly associated to sarcopenia. In multivariate analysis, VD levels (OR 0.27 (95% CI 0.08–0.89), $P=0.032$) and total body fat (OR 0.93 (95% CI 0.88–0.98), $P=0.008$) were found to be associated to sarcopenia, after adjusting for age, sex, CD4 lymphocyte count and ART.
Conclusion
Sarcopenia is a frequent condition in HIV-infected patients despite the young age, affecting to 23.5% of our population. Increased VD levels and body fat mass may protect from it. Clinical studies on VD supplementation for sarcopenia prevention in HIV-infected patients are warranted.

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Lithium-associated hyperparathyroidism: a case report

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Introduction
Lithium therapy is commonly used in bipolar disorder treatment. Alongside the increasing prevalence of goiter and hypothyroidism, the sustaining use of lithium therapy is associated with several metabolic disorders, such as hypercalcemia and hyperparathyroidism (HPT).
Case report
A 64-year-old woman with history of bipolar disorder treated with lithium for several years was referred to our department for evaluation of recurrent hypercalcemia and asymptomatic multinodular goiter (MNG).
Euthyroid MNG (benign fine needle biopsy) and a lithium-associated hyperparathyroidism (blood calcium 10.6 mg/dl (8.4–10.2); phosphorus 2.06 mg/dl (2.0–4.0) PTH 256 pg/dl (15–60)) were diagnosed. SestaMIBI scan was negative. A bone mineral densitometry showed reduced *T*-score in forearm (-2.0).
As lithium therapy was essential and could not be discontinued, we started bisphosphonate therapy (alendronate 70 mg/week) and conservative management with regular follow-up was planned.
After 1 year of follow-up she showed a severe deterioration of the HPT with increasing calcium levels (12.8 mg/dl). A new SestaMIBI scan suggested a left lower parathyroid adenoma (PTA) and was referred to surgery.
A total thyroidectomy and a left lower parathyroidectomy were performed. Histology confirmed a PTA with $13 \times 12 \times 8$ mm and a follicular hyperplasia of the thyroid gland, with multinodular goiter. After surgery, the calcium levels normalized and PTH levels decreased. She starts treatment with levothyroxine and maintained lithium therapy.
During 1 year, its calcium levels sustain normal, but PTH levels were mildly increased. She performed another SestaMIBI scan that was negative. She maintains a conservative management with regular follow up in our department.
Discussion
It is still unclear whether lithium-associated HPT causes four-gland hyperplasia or promotes the growth of pre-existing parathyroid adenomas (PTA). This leads to a discussion about what should be the best surgical approach. A four-gland exploration is frequently necessary, but excision of simple adenomas may be a valid option. Patients under lithium therapy should be closely monitored to potential HPT, attempting early diagnosis and avoiding associated comorbidities.

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P128

Late-onset hypoparathyroidism 15 years after thyroidectomy

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Introduction
Post-surgical hypoparathyroidism is a well known complication of total thyroidectomy. It may occur as transitory hypoparathyroidism that spontaneously recover within a few weeks/months and as permanent hypoparathyroidism needing long-term treatment. Only few cases of hypoparathyroidism newly diagnosed many years after surgery have been reported. We present a patient with hypoparathyroidism that became clinically evident 15 years after the thyroid surgery.
Case report
Female, 32 years old submitted to total thyroidectomy for papillary thyroid carcinoma (pT2N1bM0) in 1998 at another institution. She was referred to our consultation in September/09 for follow-up and remained clinically asymptomatic until March/12, when she was observed in the Emergency Department due to complaints of hand paresthesias and carpal spasm. Severe hypocalcemia (Ca$=$ 5.4 mg/dl (8.8–10.6), $Ca^{2+}=0.68$ mg/dl (1.15–1.35)) was diagnosed. She was treated with i.v. calcium gluconate and after resolution of signs and symptoms was discharged on calcitriol, calcium carbonate and cholecalciferol therapy. Due to constipation, she abandoned by her own initiative calcitriol a month later. The analytical study of calcium metabolism revealed postsurgical hypoparathyroidism (PTH$=$10.2 pg/ml (10.0–65.0), Ca$=$4.1 mEq/l (4.2–5.1), $Ca^{2+}=2.04$ mmol/l (2.26–2.64), $PO4^{3-}=4.6$ mg/dl (2.7–4.5), $Mg^{2+}=1.50$ mEq/l (1.55–2.05)). The patient is now asymptomatic on supplementation with calcitriol, calcium and cholecalciferol.

Conclusion
Late-onset hypoparathyroidism appearing years after total thyroidectomy is a rare condition. Symptoms of hypocalcemia may be latent and subtle (such as weakness, tiredness, irritability and depression) and thus attributed to other diseases. Although hypocalcemia typically occur after surgery, progressive atrophy of the parathyroid glands, leading to its insufficiency years after thyroid surgery, may result in a late latent hypocalcemia. Although we lack data on radioactive iodine (I^{131}) therapy and she is not able to ensure us that information, it is another possible factor contributing to late hypoparathyroidism.

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P129

Does recognition of vitamin D deficiency affect the indication for surgical treatment in asymptomatic primary hyperparathyroidism?

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Introduction
Current guidelines for the management of asymptomatic primary hyperparathyroidism (A-pHPT) recommend that 25-hydroxyvitamin D levels should be assessed in all patients and vitamin D deficiency (VDD) should be cautiously corrected. It is unknown whether VDD affects the probability to meet surgical criteria currently proposed for A-pHPT. Aim of study was to evaluate whether VDD recognition affects the probability to meet surgical criteria in A-pHPT patients.
Methods
Eighty consecutive A-pHPT patients were studied (mean $\pm$ s.d. age: 66.5 ± 8.9 years; male/female = 10/70; PTH: 179.8 ± 134.7 ng/l, calcium: 10.8 ± 0.7 mg/dl; ionized calcium: 1.39 ± 0.10 mmol/l; 25OHD: 30.0 ± 21.3 ng/ml). VDD was defined as 25-hydroxyvitamin D <20 ng/ml. The criteria for surgery of III International Workshop on the Management of ApHPT were considered, i.e.: Serum calcium >1 mg/dl the upper normal limit; creatinine clearance $<$ 60 ml/min; *T*-score <-2.5 at any site.
Results
VDD was present in 32 patients (40%). A-pHPT patients with VDD showed higher PTH ($P=0.0007$), total ($P=0.04$) and ionized calcium ($P=0.013$) and lower forearm *T*-score ($P=0.019$) compared with A-pHPT without VDD. No difference in fulfillment of any surgical criteria was present between A-pHPT patients with or without VDD (calcium criterion = 25 vs 14.6%, $P=0.2586$; eGFR criterion = 19.4 vs 21.7%, $P=1.000$; *T*-score criterion = 78.1 vs 64.6%, $P=0.2226$, respectively).
Conclusion
VDD is not rarely detected in A-pHPT and affects biochemical and densitometric features; however, the recognition of VDD does not affect the probability to meet surgical criteria proposed by current guidelines in A-pHPT. Thus, the assessment of vitamin D status, as recommended, does not affect the therapeutical choice for surgery in A-pHPT.

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Fahr's disease with dystonia: a case report

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Background
Fahr's disease is a rare degenerative disorder characterized by symmetrical and bilateral intracranial calcification. Movement disorders are the most common symptoms of Fahr's disease and dystonia is an uncommon presentation which accounts for only 8% of symptomatic patients.
Case report
A 47 years old female admitted to emergency department with involuntary movements of extremities and anxiety. Neurological examination was normal except repetetive contractions and involuntary movements mainly on left arm and leg. In her medical history subtotal thyroidectomy was performed for multinoduler goiter 28 years ago. There was no family history of neurological disease. She was consulted by neurology for insomnia and anxiety 5 years ago. Valproic acid and haloperidol were given with the diagnosis of epilepsia and insomnia. She has had involuntary movements for 2 years which worsened during the last 2 months and her life quality decreased rapidly. Her symptoms considered to be the side effect of haloperidol treatment and drug was stopped, but no improvement was observed after the discontinuation of haloperidol. On the laboratory, serum calcium was 6.8 mg/dl (normal 8.4–10.6 mg/dl), phosphate 4.8 mg/dl (normal 2.3–4.7 mg/dl), albumin: 4.1 mg/dl, parathormone was 0.25 pg/ml (normal 15–65 pg/ml). EMG showed 400–500 ms non-rhythmic bursts which is typical for dystonia. Cranial CT revealed massive calcifications involving basal ganglia, thalamus and cerebellar nuclei. The results supported the diagnosis of Fahr's disease caused by hypoparathyroidism. Serum calcium level was increased to acceptable range after calcitriol and calcium carbonate treatment and resulted in complete resolution of dystonia.
Conclusions
We present a case of Fahr's disease caused by longstanding hypoparathyroidism and manifested with dystonia, an unusual presentation of disease.Though it is rare, it is important to remember that hypoparathyroidism can be the cause of Fahr's disease accompanied by unusual neurological disorders.

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Predictive factors of postoperative hypoparathyroidism after total thyroidectomy

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Introduction
Hypoparathyroidism (hypoPT) is the most frequent complication after thyroid surgery, usually as transient hypocalcaemia. Permanent parathyroid lesion is less frequent, but it can extend hospital stay and complicate postoperative management. Several preoperative factors predicting the development of hypoPT have been identified, including advanced age, hyperthyroidism, surgical experience and others. We evaluate the role of some potentially predictive factors in our recent surgical series.
Description of methods/design
We analyze retrospectively 649 patients with total thyroidectomy from 2005 to 2011, followed up more than 1 year after surgery. Permanent hypoPT (PhypoPT) is defined by (iPTH) <15 pg/ml without treatment more than 1 year after surgery. (iPTH) between 5 and 15 pg/ml were defined as partial deficiency, whereas <5 pg/ml was considered total deficiency. Cases with spontaneous recovering of parathyroid function after a period of (PTHi) <15 were named as transient hypoPT (ThypoPT). We consider age, thyroid size (by weight), sex, presence of hyperthyroidism, central dissection and cause of surgery as variables in our study.
Results
449 patients didn't show postoperative hypoPT (70.6%), 151ThypoPT (23.3%; 87 of them normalized at 1st month) and 49 (7.55%) PhypoPT, 27 of them (4.1%) total deficiency. Significant differences among the three groups were found in three variables: thyroid weight, lower in PhypoPT (mean (s.d.): 53.5 (57.8) g vs 69.9 (46.8) and 63.3 (58.6) g in non-hypoPT and ThypoPT; *P*:0.027); fulfillment of central dissection, carrying an OR for hypoPT of 5.06 (CI: 3.10–8.28), and OR for PhypoPT vs ThypoPT: 3.08 (CI: 1.99–4.77)); and surgery reason (cytology/biopsy vs others; OR for HypoPT: 1.72 (CI: 1.22–2.41), and OR for PHyPT vs THyPT: 1.38 (CI: 1.04–1.84)). Neither age nor prevalence of hyperthyroidism showed differences among three groups as well as sex distribution.
Conclusion
Parathyroid damage is more prevalent in total thyroidectomy when it is indicated for malignant or suspicious cytology or biopsy, particularly when it is completed with dissection of central cervical compartment in smaller glands. Our series finds that gender, age or hyperthyroidism haven't got influence in the risk of postoperative hypoPT.

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Normocalcemic primary hyperparathyroidism: an Italian epidemiologic study

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Primary hyperparathyroidism (PHPT) is defined by hypercalcemia and high PTH levels. In recent years a variant of PHPT has been described, namely normocalcemic PHPT (NPHPT), which is characterized by normal serum calcium and high PTH levels, in the absence of other causes of secondary hyperparathyroidism. The epidemiology of NPHPT is poorly understood. We performed a survey in the early fall in a small Southern Italian village, in which all adult residents ($n=1811$) were invited to participate. A total of 1056 accepted to participate and blood samples were collected for measurement of serum calcium, albumin, creatinine, PTH and 25OHD. Daily calcium intake was also evaluated using a self-administered questionnaire. A complete set of results was available in 679 subjects (age 18–89 years; 422 F and 257 M). Four women (0.6%) had PHPT. Two hundred and eighty-eight individuals (42.4%) had high PTH (nl: 10–65 pg/ml) and normal albumin-adjusted serum calcium (alb-Ca; nl: 8.6–10.2 mg/dl). Two hundred and sixty-three of them were excluded because of serum 25OHD <30 ng/ml ($n=241$, 83.7%) or eGFR <60 ml/min per 1.73 m^2 ($n=22$, 7.6%). NPHPT was identified in the remaining 25 subjects (11 F (mean age 47 years, 5 postmenopausal) and 14 M (mean age 47 years), with an overall prevalence of 25/679 (3.5%). PTH, alb-Ca and 25OHD (mean $\pm$ S.D.) concentrations were 89.0 ± 21.5 pg/ml, 9.0 ± 0.3 mg/dl, and 37.6 ± 7.2 ng/ml, respectively. No relationship was found between PTH and quartile of daily calcium intake. In conclusion, the association of high PTH and normal alb-Ca is rather a common finding, and in the majority of cases is linked to low 25OHD levels and less frequently to renal failure. However, a definite proportion of subjects (3.5%) met the criteria of NPHPT. Longitudinal studies in the latter subjects is needed to establish whether NPHPT represents an early stage of classical PHPT or a separate entity.

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Plasma lipid levels in relation to the status of vitamin D sufficiency

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Introduction
Several studies have investigated a possible action of vitamin D metabolites on different lipid fractions but the possible mechanism by which they could act remain unknown, with varying results in observational and intervention studies.
Objective
To study the relation between vitamin D levels and lipids levels.
Methods
We undertook a population-based cohort study in Spain. At baseline (1996–1998), 1226 subjects were evaluated. Follow-up visits were performed in 2002–2004 and 2005–2007. At baseline and follow-up, participants underwent an interview and a standardized clinical examination. At the second visit, 25-hydroxyvitamin D levels and iPTH levels were measured. To evaluate the association between dyslipemia and 25-hydroxyvitamin D levels, the odds ratio (OR) and 95% CI were calculated using a logistic regression model. In all cases the level of rejection of a null hypothesis was $\alpha=0.05$.
Results
The mean levels of total cholesterol (TC) in second study were: 249.73 ± 53.05, triglycerides: 111.18 ± 73.21, HDL: 66.35 ± 16.38 and LDL:161.6 ± 46.71 mg/dl. In the third study, levels were 201.61 ± 38.11, 116.61 ± 78.60, 54.78 ± 12.98 and 123.58 ± 33.12 mg/dl. Mean levels of different lipid fractions in the second study depending on the presence or absence of vitamin D deficiency were: TC: 256.68 ± 51.91 vs 246.07 ± 53.37 (P 0.006), triglycerides: 87.539 ± 119.94 vs 106.34 ± 63.82 (P 0.01), HDL: 68.47 ± 17.50 vs 65.24 ± 15.67 (P 0.007), LDL: 120.91 ± 30.76 vs 108.45 ± 25.05 (P 0.001); ratio TC/HDL: 3.90 ± 0.96 vs 3.90 ± 0.98 (P 0.9); ratio LDL/HDL: 1.69 ± 0.51 vs 1.67 ± 0.53 (P 0.7). Mean levels of 25-hydroxyvitamin D were significantly lower in subjects with high TC (22.5 vs 24.2 ng/ml), high HDL (22.1 vs 23.2 ng/ml) and triglycerides (21.1 vs 23.2 ng/ml). No correlation was found between CT and 25-hydroxyvitamin D ($r=-0.06$, $P=0.08$), but there was a negative correlation with HDL and TG ($r=-0.07$, $P=0.03$ and $r=-0.09$, $P=0.01$) adjusted for age, sex and weight. In the cross-sectional study in the multivariate model we didn't obtained relationship between vitamin D deficiency (<20 ng/ml) and elevated total cholesterol (OR = 0.85, 95% CI 0.57–1.28) or low HDL (OR = 1.42, 95% CI 0.75–2.71) after adjustment for age, sex and obesity, but the relationship between vitamin D deficiency and hypertriglyceridemia persisted, so that subjects with deficit of vitamin D were more likely to have high triglycerides (OR = 0.68, 95% CI 0.47–0.98, $P=0.03$). In the prospective study, after 4 years of follow up, patients with or without vitamin D deficit, had similar risk of hypertriglyceridemia adjusted by age, sex and presence of obesity (OR: 1.01, $P=0.95$). No relationship was found with other lipid fractions in prospective study.
Conclusion
– Vitamin D deficiency is associated with increased prevalence of hypertriglyceridemia.
– Vitamin D deficiency is not related to the incidence of dyslipidemia.

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Primary hyperparathyroidism and vitamin D deficiency. therapeutic implications

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Primary hyperparathyroidism is a rather frequent disorder characterized by high plasma PTH and calcium. Vitamin D deficiency is prevalent in all areas of the world. Vitamin D deficiency has been described in patients with primary hyperparathyroidism. When present, vitamin D deficiency may be associated with large size parathyroid adenomas and musculoskeletal pain.
The aim was to describe two cases of primary hyperparathyroidism and vitamin D deficiency.
A patient, male aged 87 years, was hospitalized for coronary insufficiency and diffuse musculoskeletal pain. During hospitalization high plasma calcium was observed, calcium levels being 10.5 mg/dl. Laboratory investigations revealed high plasma parathyroid hormone levels, PTH being 117 pg/ml (normal values 10–65 pg/ml) and low plasma $25(OH)D_3$ levels, $25(OH)D_3$ being 8 ng/ml (normal values <30 ng/ml). Bone mineral density was measured in the neck of the left femur and revealed a T-score of -3.05. Vitamin D supplementation was initiated followed by the administration of alendronate. Ultrasonography revealed an adenoma beneath the right lobe of the thyroid gland. Conservative management was chosen due to old age. A patient, female aged 42 years, presented with diffuse musculoskeletal pain. Laboratory investigations revealed high plasma PTH (PTH 163 pg/ml), low plasma $25(OH)D_3$ ($25(OH)D_3$ 9 ng/ml) and calcium 11.3 mg/dl. On scintigraphy a parathyroid adenoma was visualized beneath the left lobe of the thyroid gland. Vitamin D supplementation was performed followed by surgery to remove the parathyroid adenoma. Postoperatively, the patient developed hungry bone syndrome. In both cases after vitamin D supplementation the diffuse musculoskeletal symptoms improved.
Conclusions
Vitamin D deficiency may be found in the context of primary hyperparathyroidism. When present, vitamin D supplementation should be initiated cautiously, as it may aggravate primary hyperparathyroidism. Cautious vitamin D supplementation is however necessary, will not cause an increase in calcium and PTH levels and will improve musculoskeletal pain.

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Prevalence and correlates of vitamin D deficiency in Turkish adults

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Enormous evidence suggests that vitamin D deficiency could be linked to several chronic diseases, including cardiovascular disease, diabetes, obesity, depression and cancer. The purpose of this study was to examine the prevalence of vitamin D deficiency and its correlates to test the hypothesis that vitamin D deficiency was common among adult Turkish population, particularly in women. The Turkish Diabetes, Hypertension, Obesity and Endocrine Disease Survey (TURDEP-II) Jan to June 2010 data were analyzed for vitamin D levels in adult participants ($n=9560$, mean (S.D.) age: 45.3 (15.4) years, 64% women).
Serum 25(OH)D was inversely correlated with PTH ($r=-0.122$, $P<0.001$). Serum 25(OH)D levels (controlled for age, gender, region, living environment, BMI, waist, and season) correlated with fish, cheese, and sunflower oil consumption; serum levels of creatinine, LDL-cholesterol, HDL-cholesterol, FT_4, vitamin B12, folates, IGF1, IGFBP3 and eGFR.
Vitamin D deficiency was defined as 25(OH)D concentrations $\leq$20 ng/ml ($\leq$50 nmol/l). The overall prevalence rate of vitamin D deficiency was 93%, with the highest rate seen in younger ($<$40 years) age group (96.2%) in women, and elderly ($\geq$65 years) age group (91.9%) in men.
Multiple logistic regression model with *Z* scores showed that male gender, increase in total cholesterol (39.3 mg/dl), HDL-cholesterol (12.3 mg/dl) and IGF1 levels (69.0 ng/ml) positively associated; but eGFR (18.6 ml/min) and PTH (22.7 pg/ml) levels inversely associated with vitamin D ($\geq$20 ng/ml).
In conclusion, vitamin D deficiency was common among adult Turkish population, particularly in child-bearing age women. Given that vitamin D deficiency is linked to important risk factors of leading cause of morbidity and mortality in Turkey, it is important that health professionals should be aware of this connection and health authorities arrange intervention strategies, such as vitamin D fortification of foods to correct vitamin D deficiency at the population level.

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Vitamin D deficiency in urban adult population of south-eastern Poland

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Vitamin D deficiency has been recognized as a common public health issue in many countries, however its prevalence and impact on calcium metabolism parameters has not been extensively studied in Poland yet.
The aim of the study was to assess the vitamin D status in sample of Polish urban population.
Material and methods
Study included 273 healthy volunteers (76 males, 197 females, median age 58 years) living in city Krakow in south-eastern part of Poland. 56% of them were examined during the late autumn–winter, the rest during spring–summer of 2011–2012. In each subject plasma level of vitamin 25(OH)D_3, PTH, total calcium, phosphate, alkaline phosphatase (AP), as well as urinary calcium concentration were assessed. The demographic data and dietary daily calcium intake questionnaire were also obtained.
Results
Median vitamin D level was 21.2 ng/ml (range 3.0–55.4 ng/ml). Mild vitamin D deficiency ($<$50 ng/ml) was noted in 98.9%, severe deficiency in 81% of studied population. The vitamin D level was statistically lower in winter than in summer (median value of 19.6 vs 23.5 ng/ml, $P=0.0002$; 88.2 vs 71.7% of subjects). There was statistically significant correlation between vitamin D and PTH levels ($r=-0.21$, 95% CI: -0.27 to -0.09, $P=0.0004$) and urinary calcium concentration ($r=0.15$, 95% CI: 0.02 to 0.27, $P=0.02$). Correlation between vitamin D and AP almost met statistical significance ($r=-0.12$; 95% CI: -0.23 to $+0.007$; $P=0.058$). There was no significant relationship between vitamin D and plasma calcium and phosphate levels. There was no seasonal variation in plasma PTH and calcium levels.
Conclusions
Vitamin D deficiency in Polish adult population is very frequent even during the summer. Vitamin D level in vitamin D deficient population is an important determinant of PTH level.

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The effects of lactose intolerance and lactose intolerance associated diseases on serum vitamin D levels, bone metabolism markers, bone mineral density and bone fractures

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Introduction
Lactose intolerance itself is linked with lower peak bone mass (PBM) and with decreased bone mineral density (BMD) which may decrease further with lactose intolerance associated comorbidities. The aim of our study was to examine how bone metabolic parameters are affected (change of bone metabolism markers, BMD and frequency of fractures) in lactose intolerance which was accompanied by previously diagnosed hyperlipidemia or by hypothyroidism.
Description of methods
Forty-three female patients (34.88 ± 4.4 years) with lactose intolerance were divided into three groups: $n=16$ female patients (32.8 ± 4.4 years) with lactose intolerance only (1), $n=13$ female patients (35.6 ± 4.2 years) with lactose intolerance and previously diagnosed hyperlipidemia (2) and $n=14$ female patients (36.5 ± 2.9 years) with lactose intolerance and treated hypothyroidism (3) . BMD was measured by dual-energy X-ray absorptiometry (DEXA) at the one third of distal radius, the femoral neck and the lumbar spine region (L2–L4). Laboratory analysis included routine and also calcium metabolism parameters such as: 25-hydroxyvitamin D (25-OHD_3), parathyroid hormone (PTH), β-CrossLaps, 25-OH D_3- vitamin D, osteocalcin (OC). Also prevalence of fractures was recorded. Statistical analysis was performed by ANOVA, with *post-hoc* test Bonferroni.
Results
Bone fractures, BMD and vitamin D levels differ among the groups significantly ($P<0.05$). The highest frequency of bone fractures was observed in patients with lactose intolerance and previously diagnosed hyperlipidemia (0.84/patient) which was accompanied by the lowest 25OH vitamin D level (26.08 ± 9.25 nmol/l) and bone mineral density in femoral neck region (Zsc: -1.29 ± 0.21).
Conclusion
The recognition of lactose intolerance in time, special attention for associated comorbidities (e.g. lactose intolerance with hyperlipidaemia) and choosing the proper treatment (e.g. sufficient supplementation of vitamin D) should become part of the osteological and fracture prevention of women in fertile age.

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P138

Normocalcemic hyperparathyroidism

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Introduction
In the whole world normocalcemic hyperparathyroidism (NC HPT) is an emerging entity and a growing problem. This disease is characterized by high levels of intact parathyroid hormone with normal total or ionized serum calcium. Our aim is to analyze its clinical and biological profile and its etiologies.
Subjects and methods
We have studied 18 subjects with NC HPT. They all had routine analyses, plasma calcium and phosphorous, and assessment of parathyroid hormone (PTH) and vitamin D. kidney and bone explorations were done too.

Results
In this group, 16 were females (88.8%) and 2 were males. Their mean age was equal to 51.2 years (36–70), their mean plasma calcium = 95 mg/l, mean phosphorous = 32 mg/l, mean PTH = 223 pg/ml ($\leq$60). The consultation motive was bone problems in 33.3%. Bone mineral density was low or very low in 14 (77.7%). Recurrent kidney stones were observed in three cases = 16.6%, renal insufficiency = 0%. For etiologies NCHPT was due to vitamin deficiencies in 57.1% and to primitive hyperparathyroidism in 42.9%.
Conclusion
As in classical HPT, NC HPT is prevailing in female cases. In NC HPT bone lesions are more frequent than kidney problems. For etiologies, vitamin D deficiency is slightly more frequent than idiopathic primary HPT, but large series are necessary to confirm our results.

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Familiar hypocalciuric hypercalcemia a rare cause of hypercalcemia

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Introduction
Familial hypocalciuric hypercalcemia (FHH) is caused by inactivating autosomal dominant mutations with high penetrance of CaSP gene. Contrary to severe neonatal hyperparathyroidism, caused by homozygous inactivation of the gene, familial hypocalciuric hypercalcemia is usually associated with inactivating variants in heterozygoty.
Case
Male patient, 73 years, with history of Behçet's disease and pulmonary sarcoidosis, was refered for evaluation of hypopituitarism. During the evaluation phospho-calcium metabolism abnormalities were detected: calcium 5.5 mEq/l (4.05–5.2), ionized calcium 2.88 mEq/l (2.26–2.64) inorganic phosphorous 2.8 mg/dl (2.7–4.5), magnesium 1.86 mEq/l (1.55–2.05), confirmed in further studies, intact PTH of 88.9 pg/ml (10.0–65.0), with subsequent normal values. Urinary calcium to creatinine ratio was 0.004 (urinary calcium 2.2 mEq/24 h; plasma creatinine 1.3 mg/dl; urinary creatinine 1164 mg/24 h). Parathyroid ultrasound and scintigraphy revealed no changes as well as bone densitometry. The high calcium, the inappropriately high/normal intact PTH levels and the low urinary calcium, associated to the absence of symptoms of hyperparathyroidism, prompt us to consider the diagnosis of FHH and to request genetic study. It revealed the presence, in heterozygosity, of the pathogenic mutation c.1311C > A (p.Cys437X), in exon 4, of CaSR gene. Study of descendents and siblings was recommended, stressing the benign nature of this entity.
Discussion
Familial hypocalciuric hypercalcemia is usually asymptomatic and characterized by mild to moderate hypercalcemia, relative hypocalciuria, normal intact PTH, which can be mildly elevated in 20% of the cases, and high-normal levels of magnesium. Urinary calcium to creatinine ratio $<$0.01 is found in $\sim$80% of individuals with FHH, while a similar proportion of cases of primary hyperparathyroidism have levels higher than this. FHH is a benign entity and usually does not require treatment. The importance of diagnose and screening is due to the need of differential diagnoses, especially with primary hyperparathyroidism, in order to avoid unnecessary therapeutic interventions.

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P140

Vitamin D deficiency in lithuanian school graduates females

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Background
There are evidences that young adults are at risk for poor vitamin D nutritional status. The objective of this study was to investigate the level of vitamin D in school graduates females and the factors associated with vitamin D status.
Material and methods
School graduates females from three Lithuanian cities were enrolled into the project from March to June of 2012. General health, nutrition, physical activity status was assessed by questionnaires. Height, body mass were measured. Blood samples were taken for complete blood count and 25(OH)D. Information on average number of sunshine hours every month in particular city was obtained from the meteorological service.
Results
The study included 287 young adult females. Mean age was 18.33 $\pm$ 0.62 years, mean BMI 21.09 $\pm$ 2.91 kg/m^2. 85 females used solarium. Their 25(OH)D level was 22.29 $\pm$ 7.42 ng/ml, significantly higher than those who has not used solarium. Data of females using solarium have been excluded from further analysis.
Mean 25(OH)D concentration in 202 subjects was 15.72 $\pm$ 7.17 ng/ml. The prevalence of vitamin D deficiency (serum 25(OH)D $<$20 ng/ml) was 73.8%. 22.8% of females were found to be vitamin D insufficient (25(OH)D level 20–30 ng/ml) and only 3.4% young adults females were vitamin D sufficient. There were no differences in time spent outdoors, sleep, rest and physical activity hours, meat, fish and milk consumption between groups according to vitamin D status. Regression analysis showed significant 25(OH)D level and sunshine hours relationship ($B = 2.64$, $P < 0.0001$). Mean 25(OH)D level in April was 13.71 $\pm$ 6.10 ng/ml, in May 18.74 $\pm$ 6.72 ng/ml and in June 22.27 $\pm$ 8.67 ng/ml ($P < 0.0001$). Regression analysis revealed relationship between 25(OH)D level and white blood cells count ($B = 0.921$, $P = 0.003$) and platelets count ($B = -0.020$, $P = 0.028$).
Conclusions
Vitamin D deficiency and insufficiency are highly prevalent in school graduates females. Sunshine exposure is the main source of vitamin D. WBC count increase, if vitamin D level increases.

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Does vitamin D status predict handgrip strength in young adult women?

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Introduction
Increasing evidence suggesting an integral role of vitamin D in skeletal muscle function. The relationship between muscle strength and vitamin D status has been investigated to some degree in older populations, however there has been very little research in younger adult women. This study aims to measure the effects of vitamin D on handgrip strength in young adult women.
Method
A total of 141 healthy women with mean age of 33.8 $\pm$ 6.8 were included in this cross-sectional study. Vitamin D, calcium (Ca), phosphorus (P) and parathormon (PTH) levels were measured. Dominant and non-dominant hand-grip strength were measured with hand-grip dynamometer for three times, To minimize seasonal variations all measurements were taken in the March. Ethical committee approved the study protocol.
Results
Mean vitamin D levels and mean handgrip strength were 19.5 $\pm$ 2.96 ng/ml and 32.6 $\pm$ 5.7 kg respectively. There was no significant difference between the mean handgrip strength of women with vitamin D deficiency (31.9 $\pm$ 5.2 kg) or vitamin insufficiency (32.3 $\pm$ 5.9 kg) and women with normal vitamin D levels (32.2 $\pm$ 6.6 kg). In correlation analysis; there was no significant relation between the mean handgrip strength and the vitamin D, Ca, P levels. There was weak, but significant correlation between the PTH levels and mean handgrip strength ($r =$ 0174; $P = 0.049$).
Conclusion
Our results suggest that vitamin D status does not predict handgrip strength at least in young adult women.

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P142

Influence of preoperative characteristics in the result of Tc99m-sestaMIBI scan in patients with primary hyperparathyroidism

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Primary hyperparathyroidism (PHP) is a common endocrine disorder. It is due to a single parathyroid adenoma in 80% of cases, most of the remainder due to hyperplasia of all four parathyroid glands. The only curative procedure is parathyroidectomy, but preoperative localization can be difficult. Isotope scanning with sestaMIBI is considered to be useful in localizing any parathyroid adenoma to help guided surgery. The aim of this study is demonstrated the influence of biochemical parameters, gland size and thyroid disease in accuracy of MIBI scintigraphy in the localization of parathyroid adenomas.
A series of 196 patients with PHP who underwent parathyroidectomy from 1995 to 2012 was included and divided into two groups: positive MIBI and negative MIBI. Positive value was defined when the pathologic gland was correctly localized and negative value when discordant result or no gland was localized.

	MIBI+ (124 cases)	**MIBI**− (72 cases)
Age	64 (33–84)	63 (21–84)
Female/male	77.4/22.6%	83.3/16.7%
Nephrolithiasis	40.3%	39.4%
Osteoporosis	25.8%	30.6%
Thyroid disease	22.6%*	40.3%*

*$P < 0.05$.

Except one case of parathyroid carcinoma all were adenomas in MIBI+ group, however in MIBI− group, there were five hyperplastic glands and three cases of double adenoma, rest (86.1%) were adenomas. Serum calcium, phosphate, urinary calcium, PTH and 25OH-vitamine D values were similar and there was no statistically difference between size and weight of the pathologic gland in both groups.
Conclusion
This retrospective study shows there is no influence of biochemical parameters, gland size or tumoral weight in the accuracy of MIBI scintigraphy. We also demonstrate the negative effect of simultaneous thyroid disease in this diagnostic thecnique. More studies are needed to evaluate the influence of other parameters, like cell type, in accuracy of MIBI scan in parathyroid adenomas.

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Cystic parathyroid adenomas: ultrasonography features

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Introduction
Parathyroid adenomas appear typically as homogeneous, hypoechoic lesions compared to the thyroid on the ultrasonography examination. They can be detected easily when they are more than 1 cm. However, cystic parathyroid adenomas are rare. When present, they appear as decreased echogenic areas in the gland. Extrathyroidal feeding from an artery at one pole of the gland is characteristic which is seen on Doppler imaging. We aimed to present a case series including seven cases in which glands looked partially cystic and heterogenic from the 88 primary hyperparathyroidism patients presented to our clinic.
Materials and methods
Eighty-eight patients who were diagnosed with primary hyperparathyroidism between January 2010 and September 2012 were reviewed retrospectively. All the cases were diagnosed with primary hyperparathyroidism in the endocrinology outpatient clinic for room surgery was planned. All the cases were confirmed to have primary hyperparathyroidism histologically. Sonographic images and reports were obtained from the archives and they were reanalyzed. All of the ultrasonography images were taken by high resolution superficial tissue probes with 13 MHz (Hitachi EUB 7000, Tokyo, Japan). The inside of the lesions looked frequently highly hypoechoic and homogeneous but less hypoechoic compared to solid and strap muscles. Six cases which had atypical appearance with a lesion that was heterogeneous and included cystic areas were separated and investigated further. Demographic features of those patients were as follows: mean age was 51 ± 14.5 and all of them were female. All of these cases were sent to ultrasonography because of suspicion of a parathyroid lesion based on clinical and laboratory evidence including elevated parathyroid hormone and or high serum calcium values. On the ultrasound, five of the lesions were located on the left and one was located on the right and they were frequently observed postero-inferior to the thyroid lobe on the left. Sonographicly, the longitudinal dimention of the adenomas were more than 2 cm (by dimention: 2.8 ± 0.7). Mean PTH: 185 ± 38, $Ca = 10.9 \pm 0.8$. Both mean PTH and calcium were elevated. In histopathologic examination all cases were diagnosed with parathyroid adenoma.
Conclusion
Cystic parathyroid adenomas are presented in the literature as case reports. Cystic parathyroid adenomas were more frequently detected in our clinic then reported in the literature (7%), and clinically our cases had higher PTH and calcium values and a larger dimentions compared to the cases reported in the literature. Based on these features our patients may need to be evaluated as a separate group.

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P144

Primary hypoparathyroidism and autoimmune endocrine disorders

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Introduction
Primary hypoparathyroidism (PH) is a rare condition. After surgery and chronic alcoholism, an autoimmune disease is the most common etiology and must specifically be considered in the context of a patient with other autoimmune endocrine diseases.
Case report
MASD a male caucasian patient aged 31, was admitted to the emergency department because of perioral and hand paresthesias, and carpal spasm, in the context of a generalized anxiety reaction. A previous diagnosis of Hashimoto's thyroiditis treated with levothyroxine was reported. There were no other known diseases nor previous surgeries, alcohol consumption or drug use. Family history was unremarkable as it was physical examination, except for positive Chvostek and Trousseau signs. Analytical evaluation revealed hypocalcaemia with increased serum phosphate and normal albumin and arterial pH; hypercalciuria was found. Serum PTH was low with normal 25-hydroxyvitaminD levels and normal parameters of bone formation and reabsorption as well as normal bone densitometry. Thyroid function was normal under therapy. Normal adrenal and pituitary function were found. Antibodies against parathyroid cells were negative. Genetic testing revealed the c.2968A>G(p.Arg990Gy) polymorphism at exon 6 of the calcium sensing receptor (CASR) gene.
Discussion
PH is a very rare condition. In this patient, the presence of known Hashimoto's thyroiditis made autoimmune PH the most likely diagnosis, suggesting a polyglandular autoimmune disease. However parathyroid auto-antibodies were negative and a specific polymorphism of the *CASR* gene was found that according to published data may be associated with gain of function of calcium sensor. Reported prevalence of this polymorphism is so low, that it must be considered causative in this patient. In conclusion in this young patient with autoimmune thyroid disease, PH was found in relation to a specific polymorphism of the *CASR* gene. A rose is not always a rose.

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Experience in the treatment of primary hyperparathyroidism with cinacalcet: data after 12 months of treatment

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Introduction
Cinacalcet is an oral calcimimetic indicated in treatment of primary hyperparathyroidism if patients do not accept surgery, do not carry out the surgical criteria, there is failure of previous surgery or serious comorbidity that makes surgery impossible.
Methods
Descriptive study that included 20 patients with primary hyperparathyroidism who had complete at least 12 months of treatment with cinacalcet for different reasons (eight patients refusal to parathyroidectomy, three surgery not possible due to comorbidities and nine progressive hypercalcemia prior to surgery). We recorded clinical and biochemical data at baseline, and after 3, 6 and 12 months of treatment.
Results
After 3 months of therapy, serum calcium was significantly decrease (10.7 vs 11.73 mg/dl, $P<0.001$) and serum phosphorus was significantly increased (2.62 vs 2.41 mg/dl, $P=0.004$) while no significant change occurred in serum PTH (181.91 vs 195.47 $P=0.695$). No further variation was observed after 6 months as compared to 3 months follow up. After 12 months serum PTH was significantly decreased as compared to baseline (152.47 vs 181.91, $P=0.028$) as well as serum calcium (10.2 vs 11.73 mg/dl, $P<0.001$) and phosphorus levels (2.71 vs 2.41 mg/dl, $P=0.01$). Normocalcemia (S-Ca <10.1 mg/dl) was achieved in 55% of patients.
Usually the medication was well-tolerated. Most common adverse events were nausea and vomiting, especially at the beginning of therapy.
Conclusion
Hypercalcemia is rapidly improved by cinacalcet and remains stable after 12 months follow-up. Cinacalcet is an effective alternative in non-surgical treatment of primary hyperparathyroidism in patients with recurrent disease or in case of surgical contraindications. Furthermore, cinacalcet may be useful in the preoperative hypercalcemia management.

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P146

Primary hyperparathyroidism in people under 30

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Introduction
Primary hyperparathyroidism (HPT) is usually observed in old people. It is very rare in children and young adults. Our aim is to analyze clinical, biological aspects, and outcome in six subjects under 30.
Methods
The six subjects were chosen among 36 people hospitalized for primary HPT from 2009 to 2012. All had clinical examination, routine analyses, biological (blood and urinary calcium and phosphorus, and vitamin D) and hormonal (at least three PTH) assessments. Radiological exploration was based on cervical ultrasound and MIBI scintigraphy. After medical treatment of severe hyper calcaemia, they were all operated on, and followed.
Results
Mean age = 24 (18–30). Primary HPT seemed to be sporadic. Our patients suffered mainly from bone disorders (five cases) and from symptoms of severe hyper calcaemia ($n=1$). Mean calcaemia = 131.5 mg/l (85–110 mg/l), mean phosphorus = 23.8 mg/l (25–45 mg/l), mean PTH = 753.5 pg/ml (16–87 pg/ml). Calcaemia was ≥ 140 mg/l in two. Ultrasounds and MIBI showed a single parathyroid tumour in all of them. Histological study demonstrated five adenomas and one carcinoma. The tumour size was ≥ 2 cm in all cases. Research for MEN was negative. Genetic study could not be done for socio-economical problems. Five did not relapse for 1–7 years; however one was lost in sight.
Conclusion
Primary HPT is rare in people under 30. It is more symptomatic and more severe than in elderly, but the outcome seems better even for the case classified as malignant by histology as there is not any relapsing or metastasises for more than 7 years. In this study the single parathyroid lesion seemed to be sporadic as we excluded MEN syndrome, but an isolated familial form is not totally excluded as genetic study could not be done.

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An extensive precipitation of calcium in subcutaneous tissue in patient with juvenile dermatomyositis

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Introduction
Juvenile dermatomyositis may be associated with advanced calcinosis but the mechanism that leads to their development remains unclear.
Case report
A female patient admitted to the hospital at the age of 17 for skin rash, malaise and pain in peripheral joints and muscles. Congenital bilateral hip dysplasia. After evaluation a syndrome overlap with dominant atypical dermatomyositis and SLE was suspected and treatment with prednisolone and choroquine initiated. Six months after, subcutaneous calcium deposits appeared, first in gluteal region, than polytopically. No metabolic disorders were observed; levels of PTH, phosphorus and calcium have always been in reference range, ANA and anti-dsDNA occasionally elevated.
During the last 12 years, different immunosuppressive treatments: methotrexate, cyclophosphamide, azathioprin, mycophenolat, colchicine, intravenous bisfosfonates, imunoglobulins and calcium antagonists were given in attempt to stop spreading and reduce a calcinosis but without any improvement.
Presently, patient has a Gottrons papules on MCP and PIP joints of both hands. A very prominent extensive subcutaneous calcinosis with a few skin ulcers (from which calcium is leaking) is over thorax, abdomen, proximal parts of both hands, legs and pelvis, emphasized in lateral part of a right hip. The hands, distal parts of both legs and feet are spared. The muscles are atrophic and thin. Third muscle biopsy showed no calcifications but an increased number of macrophages in the perimysium, a few CD3 T-cells in the endomysial areas.
According to the early onset and a widespread calcinosis, a diagnosis of juvenile dermatomyositis is established. Last year, Hashimoto thyreoiditis and interstitial lung disease are diagnosed.
Sedimentation rate remains elevated which, with a current health condition, despite the prolonged treatment, indicates that disease is in active phase. Lastly, a treatment with abatacept (alternatively rituximab) was recommended.
Conclusion
Revealing a pathogenesis of calcium deposits would certainly bring a new insight to this case.

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Measurement of 25-hydroxyvitamin D: evaluation of the new DIAsource ELISA assay

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Background
Vitamin D is an important contributor to musculoskeletal health and its potential involvement has recently been underlined in several non-skeletal diseases. Measurement of circulating levels of 25-hydroxyvitamin D (25OHD) represents the most reliable assessment of vitamin D status. Several assays are available but are not commutable because of a lack of of standardization. The aim of our study was to evaluate the performance of a new ELISA for measurement of 25OHD levels.
Methods
25OHD levels were measured with a newly released ELISA (Diasource Immunoassays), a simplified method without pre-treatment step. Method comparison was performed using 199 patients' samples with automated chemiluminescent immunoassay commonly used in clinical laboratories (Liaison, DiaSorin).
Results
According to our automated assay routine cut-points, serum concentrations of 25OHD were below 20 ng/ml in 96 patients, between 20 and 30 ng/ml in 68 patients and above 30 ng/ml in 35. The ELISA and the automated methods

were significantly correlated ($r=0.9230$; $P<0.0001$) and the Passing and Bablock regression analysis showed a slope of 1.218 (95% CI: 1.1434 to 1.2944) and an intercept of −1.8775 ng/ml (95% CI −3.2694 to −0.3363). A small negative difference (2.7905 ng/ml; 95% CI −3.4546 to −2.1265) was highlighted, for the ELISA method through the Bland and Altman plot and important discrepancies (higher than 10 ng/ml) were observed in 12 samples.
Conclusions
Our preliminary results showed that the new 25OHD ELISA assay demonstrated a good agreement with a commonly used assay. A small difference was evidenced but its clinical impact is limited. However, additional investigations will be required to confirm the performances of this new simplified ELISA assay.

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P149

Persistent primary hyperparathyreoidism

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Introduction
Achievement of stable normocalcemia (88–98.8%) is considered a criterium of efficiency of surgical operations for primary hyperparathyroidism (PHPT) (P.Goudet *et al*. 2001, Zeiger 1997). In 1–15% of cases, persistence of PHPT is noted (Mariani *et al*. 2003, Mariette *et al.* 1998).
Description of methods
In 110 of 175 patients (aged 14–72 years), parathyroid adenoma was verified, in 57 – hyperplasia, and in 8 – parathyroid cancer. All patients underwent determination of total and ionized calcium levels, blood parathyroid hormone (PTH) levels, ultrasound imaging, PTG scintigraphy with ^{99m}Tc-sestamibi or single photon emission computed tomography (SPECT) as well as roentgen computed tomography.
Results
In seven patients, persistent PHPT was revealed due to insufficient operation volume in parathyroid hyperplasia. During first operation, one pathological gland was removed in four patients, two glands – in two, and three – in one patient. Temporary positive effect was noticed in all patients (decrease of PTH and calcium levels). In two patients, hyperplastic glands missed during first operation were located in anterior mediastinum. In one patient, three pathological glands were removed and repeated operation included sternotomy and removal of one additional hyperplastic ectopic gland from thymus. In other (female) patient, two hyperplastic parathyroid glands were removed during first operation and during repeated operation – one additional ectopic gland from the thymic peduncle. In two patients, repeated operations were associated with unrevealed parathyroid ectopia into the paraesophageal space. In the other female patient, only one parathyroid gland was removed during first operation; the other neoplasms removed as PTG turned out lymphatic nodes. During repeated operation, three hyperplastic parathyroid glands were removed.
Two female patients are followed up in ambulatory. In one of them, in addition to one hyperplastic parathyroid gland, two neoplasms taken first for PTG were removed but then were verified histologically as lymphatic nodes. In the second of them, two parathyroid adenomas were removed but histologically they turned out hyperplastic PTG. Now both patients are under additional examination.
Conclusion
PHPT persistence is caused by inadequate operation volume in cases of PTG hyperplasia. The special difficulty of intraoperative differential diagnostics of morphological PHPT forms is due to the fact that, in cases of hyperplasia, parathyroid glands become hypoplastic asynchronously, and in cases of hyperplasia, they become hyperplastic also non-simultaneously. Biopsy of visually non-enlarged PTG is necessary. Observation of double parathyroid adenomas is either mistaken or they are casuistically rare.

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Prevalence of multiple endocrine neoplasia type 1 syndrome in primary hyperparathyroidism

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Objective
To assess the prevalence of multiple endocrine neoplasia type 1 (MEN1) in patients with symptomatic primary hyperparathyroidism (PHPT).
Methods
A retrospective analysis of 75 consecutive patients with symptomatic PHPT from January 1994 to July 2012 was done, who underwent parathyroid surgery at our centre. Five patients had MEN1 syndrome. Among them 1 was familial MEN1. The patients with MEN1 were analyzed based on clinical presentation, biochemical and hormonal profile, imaging modalities and treatment outcome.
Results
Mean age of the study patients was 28.6 ± 12.9 years (male:female = 4:1). Mean age of the rest all patients was 43.5 ± 11.5 years. Four were symptomatic at presentation and one was diagnosed on family screening. Mean duration of symptoms was 23.8 ± 12.1 months. Bone pains and painful proximal myopathy were the commonest presentation (4/4), followed by pathological fractures in one case. Distal renal tubular acidosis was diagnosed in one case, which normalized after surgery. The most common presenting manifestation was PHPT in four patients (80%), followed by hyperprolactinemia due to pituitary tumor in one patient (20%). PHPT was a universal feature (100%) in all MEN1 syndrome followed by pituitary tumors in three cases (60%) and enteropancreatic neuroendocrine tumors in two cases (40%), with both being insulinoma. Among the pituitary tumors, prolactinoma and nonfunctioning pituitary adenoma were present in two each cases demonstrating equal prevalence.
All PHPT patients underwent parathyroidectomy and the ones with MEN1 had mean parathyroid gland weight was 1235.6 ± 684.5 mg, which was larger than the rest (mean parathyroid gland weight was 835.4 ± 178.5 mg, $P=0.04$). Three PHPT patients with MEN1 syndrome had double adenoma and two patients had multiglandular parathyroid involvement.
Discussion
PHPT patients with MEN1 tend to be younger with multifocal involvement and larger glands. Eighty % of MEN cases had PHPT as initial manifestation followed by hyperprolactinemia in 20% cases. Our series demonstrated higher incidence of symptomatic PHPT, higher prevalence of pituitary tumors (80%) and insulinoma (40%). Additionally prevalence of pituitary involvement (80%) outscores the prevalence of enteropancreatic neuroendocrine tumors (40%). These deviations from classic involvement depicted in literature could be due to small sample size of the study population.
Conclusion
All young patients with double adenoma or multiglandular parathyroid involvement should be screened for MEN1 syndrome irrespective of the symptoms. To avoid the recurrent surgical procedure, high index of suspicion is needed for diagnosis.

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P151

Does the severity of vitamin D deficiency affect the prevelance of gastrointestinal polyps?

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Introduction
The beneficial effects of vitamin D against gastrointestinal polyps and cancer in the gastrointestinal system as a whole remain unknown and relevance in repeat populations remain contraversial. Also in most studies the role of accompanying hypocalcemia itself is often neglected. For this reason we included only patients with low vitamin D levels and investigated the presence of gastric and colonic polyps.
Methods/design
Between March 2011 and September 2012, we investigated 25(OH) vitamin D levels in patients with clinical suspicion.We then performed colonoscopy and/or endoscopy in patients having clinical indications for these procedures.
Results
A total of 301 patients (175 female, 126 male) were divided into three groups according to their serum 25(OH) vitamin D levels: group 1: 0–10, group 2: 10–20, group 3: 20–30. 246 patients underwent colonoscopy and endoscopy while 55 cases underwent only colonoscopy. There was a total of five cancer cases (two colon, three gastric cancer) and four of these cases belonged to group 1 and one patient to group 2. We found colonic polyps in 29, 44, and 8 cases in groups 1, 2, and 3 respectively.The difference between the groups 1, 2, and 3 by means of polyp presence was significant. We found polyps by upper endoscopy in only 12 cases. We found a significant correlation between colonic polyps and increased

age, high Bun and creatinine levels and increased visceral fat but not with BMI, body fat percent, *H.pylori* presence, SES or SCS. But both endoscopic and colonoscopic polyp presence was found to be significantly increased in diabetic patients.
Conclusion
The severity of vitamin D deficiency is important for both stomach and colon cancers. We emphasize the importance of diabetes mellitus and visceral fat in the prevalance of endoscopic and colonic polyps.Further studies with larger number of patients are needed to confirm our results.

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P152

Vitamin D deficit and metabolic syndrome

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Background and aims
To assess the relationship between 25-hydroxyvitaminD levels and prevalence and incidence of metabolic syndrome.
Methods
We undertook a population-based cohort study in Spain. At baseline (1996–1998), 1226 subjects were evaluated. Follow-up visits were performed in 2002–2004 and 2005–2007. At baseline and follow-up, participants underwent an interview and a standardized clinical examination with an oral glucose tolerance test in those subjects without known diabetes. At the second visit, 25-hydroxyvitamin D levels and iPTH levels were measured
Results
The prevalence of metabolic sýndrome (IDF criteria) at the second and third visit was 29.4 and 42.5% respectively. Mean levels of 25-hydroxyvitamin D were lower in subjects with metabolic syndrome: 21.7 ± 6.21 vs 23.35 ± 6.29 ng/ml, $P = 0.001$. The prevalence of vitamin D deficiency (25-hydroxyvitamin D ≤ 20 ng/ml) at the second evaluation was 34.7%, with significant differences between subjects with and without metabolic syndrome (34.6 vs 26.5%, $P = 0.01$). Men with vitamin D deficiency more frequently had hypertension and metabolic syndrome than men with normal levels. Women with vitamin D deficiency had more frequently hyperglycemia, hypertension, increased waist circumference e hypertriglyceridemia. The number of present criteria was related to the prevalence of vitamin D deficiency so that for 1, 2, 3, 4, and 5 criteria the prevalence were: 31.7, 32, 33.5, 55.4, and 100%. In prospective study, 25-hydroxyvitamin D values ≤ 20 ng/ml were not significantly associated with an increased risk of developing metabolic syndrome in the next 4 years (OR = 0.99, 95% CI 0.57–1.7, $P = 0.97$) after adjusting by sex and age.
Conclusions
Vitamin D deficiency is associated with an increased prevalence but not with increased incidence of metabolic syndrome.

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P153

Vitamin D deficiency predicts incidence of obesity

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Background and aims
To assess the relationship between obesity and the incidence of hypovitaminosis D and investigate the relationship between 25-hydroxyvitamin D levels and incidence of obesity.
Methods
We undertook a population-based cohort study in Spain. At baseline (1996–1998), 1226 subjects were evaluated. Follow-up visits were performed in 2002–2004 and 2005–2007. At baseline and follow-up, participants underwent an interview and a standardized clinical examination with an oral glucose tolerance test in those subjects without known diabetes. At the second visit, 25-hydroxyvitamin D levels and iPTH levels were measured.
Results
The prevalence of obesity (BMI ≥ 30 kg/m^2) at the three visits was 28.1, 36.2, and 39.5% respectively. The prevalence of vitamin D deficiency (25-hydroxyvitamin D ≤ 20 ng/ml) at the second evaluation was 34.7%. Neither obesity at baseline (OR = 0.98, 95% CI 0.69–1.40, $P = 0.93$) nor the development of obesity between baseline and the second evaluation (OR = 0.80, 95% CI 0.48–1.33, $P = 0.39$) were significantly associated with vitamin D deficiency at the second evaluation. In subjects who were non-obese (BMI < 30 kg/m^2) at the second evaluation, 25-hydroxyvitaminD values ≤ 17 ng/ml were significantly associated with an increased risk of developing obesity in the next 4 years (OR = 2.35, 95% CI 1.03–5.4, $P = 0.040$).
Conclusions
Vitamin D deficiency is associated with an increased risk of developing obesity.

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P154

Vitamin D deficiency in morbid obesity before and after bariatric surgery

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Rationale
Patients with morbid obesity may have vitamin D deficiency and bariatric surgery may exacerbate it due to various factors such as lack of dietary compliance, reduced intake, malabsorption, etc.
Objective
To study the prevalence of vitamin D deficiency in patients with morbid obesity before and after bariatric surgery and its relationship with other laboratory parameters.
Methods
A retrospective study of 72 morbidly obese patients before and 1 year after surgery (bypass or sleeve). We collected data about: age, sex, previous comorbidities, physical examination, impedance testing, and laboratory parameters (25-hydroxyvit D, iPTH, Ca, HbA1c, total cholesterol, LDL, HDL, triglycerides, uric acid, and leptin).
Results
The mean age of patients was 45.84 ± 10.39 years, 78.4% women. Pre-and postoperatively BMI: 51.51 ± 8.11 and 33.36 ± 5.21 (P 0.00). One year after surgery there were significant decreases in HbA1c, TC, LDL, triglycerides, uric acid, leptin, and HDL increase. Mean serum 25-OH vit D pre and post were: 16.96 ± 7.87 vs 22.53 ± 12.44 (P 0.013) and iPTH: 16.9 ± 43.97 vs $41.79 \pm 1, 89$ (NS). The percentage of vitamin D deficiency (< 20 ng/ml) were pre and post: 71.8 vs 44.9% (P 0.04) and hyperparathyroidism 19.4 vs 14.6% (NS). Vitamin D levels negatively correlated with preoperative age, body fat percentage and iPTH. Vitamin D levels negatively correlated with postoperative BMI, leptin, total cholesterol, and HbA1c.
Conclusions
Patients with morbid obesity have vitamin D deficiency in 71.8% of cases preoperatively, which should lead us to measure 25OH vitamin D levels routinely in patients with morbid obesity.
After the surgical procedure, despite supplementation with vitamin D, patients maintain a high percentage of vitamin D deficiency (44.9%) and elevated iPTH.

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P155

The association of low serum 25-hydroxyvitamin D and serum osteocalcin levels with the metabolic syndrome in females with polycystic ovary syndrome in Al-Baha region, Saudia Arabia: a case–control study

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Background
Polycystic ovary syndrome (PCOS) is a common endocrine disorder in females of reproductive age. It is frequently associated with metabolic disturbances and insulin resistance, that occurs in about 50–70%. Evidences were reported about the association between vitamin D and osteocalcin with cardiovascular risk factors, regulation of insulin secretion and sensitivity.
Objectives
Evaluation of the association between of low 25-hydroxyvitamin D (25(OH) D) and serum osteocalcin levels and the components of metabolic syndrome (MS) in women with PCOS in Al-Baha region, Kingdom of Saudi Arabia (KSA)
Methods
Sixty patients with PCOS were chosen from the attendants of the Endocrinology out-patient clinic in KFH at Al-Baha, KSA. Standard metabolic, endocrine, and

anthropometric measurements were evaluated for all patients, including 25(OH) D and osteocalcin levels and oral glucose tolerance tests
Results
The prevalence of 25(OH) D deficiency <30 ng/ml was 60%. Seventeen patients (28%) showed association between MS and PCOS. Patients with MS had significantly lower level of serum 25(OH) D, osteocalcin, and CRP ($P<0.0001$). Patients with low serum 25(OH) D, had significantly higher age, weight, BMI, waist and hip circumference (WHR), and systolic blood pressure (SBP), 1-h glucose, homeostatic model assessment-insulin resistance (HOMA-IR), fasting insulin, triglycerides, and CRP. They had significantly lower levels of HOMA-β, quantitative insulin sensitivity check index (QUICKI), and HDL cholesterol ($P<0.00$ for all). Ostecalcin levels had significantly negative correlation with SBP and diastolic blood pressure (DBP), CRP, BMI, fasting and 1-h glucose, triglycerides and HOMA-IR ($P<0.00$, 0.01, 0.003, 0.000, 0.000, 0.000, 0.00, and 0.000 respectively). It has significant positive correlation with QUICKI, and HDL. In multivariate regression analysis, 25(OH) D was an independent predictors of HOMA-IR and QUICKI ($P<0.05$). Fasting insulin, SBP, and HDL were significant and independent predictors of serum osteocalcin.
Conclusions
Low levels of 25(OH) D and osteocalcin were associated with the features of MS in PCOS women. Large intervention trials are warranted to evaluate the effect of vitamin D supplementation in these cases.
Key words
25(OH) D, osteocalcin, MS, PCOS.
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P156

MSIA–SRM assay for parathyroid hormone and vitamin D binding protein: correlation with clinical immunoassay methods and application to clinical samples

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Parathyroid hormone is involved in calcium homeostasis through interactions with vitamin D. Because intact and truncated forms of PTH vary in their biological activity, assays that can accurately quantify the ratio of intact hormone to its fragments are of increasing significance in the diagnosis of endocrine, renal and bone diseases. Vitamin D and its metabolites circulate tightly bound to vitamin D-binding protein (DBP). Because DBP concentrations are altered in pregnancy, liver and renal diseases and also show genetic variations in different ethnic groups, total vitamin D in serum can be misleading. In addition, both calcium and vitamin D metabolites can decrease the secretion of parathyroid hormone (PTH).
Previously, we developed multiplexed mass spectrometric immunoassay (MSIA)–SRM assays for PTH that allow quantification of four fully-tryptic monitoring peptides (that span the entire PTH sequence) and two semi-tryptic variant specific peptides (1). Using this approach, it is possible to monitor intact PTH and also the degree of N-terminal fragmentation.
In this study, the objective was to develop a multiplexed, MSIA–SRM-based targeted assay for PTH and DBP. We applied this MSIA–SRM assay and a commercially available immunoassay to a cohort of 500 clinical samples from a variety of different patient groups including renal disease, cancer, vitamin D deficiency and other conditions that can affect calcium homeostasis. The results demonstrated excellent assay linearity ($R^2=0.90$–0.99) with sensitivity for analytes in the published clinical ranges and limits of detection in the pg/ml range. Comparison of the PTH MSIA–SRM assay with the commercial ELSA assay demonstrated good correlation in normal subjects but important differences in renal failure. There were also some unusual fragments seen in clinical samples, not previously reported in the literature.
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P157

Vitamin D deficiency, secondary hyperparathyroidism and it's influence on bone mineral density

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Introduction
The consequences of vitamin D deficiency are secondary hyperparathyroidism and bone loss, leading to osteoporosis and fractures, mineralization defects, which may lead to osteomalacia in the long term, and muscle weakness, causing falls and fractures.
Objectives
The aim of the research was to determine the frequency of vitamin D deficiency, secondary hyperparathyroidism and it's influence on bone mineral density in people of different ages.
Methods
There were examined 683 patients aged 20–94 years. 25(OH)D and iPTH level was evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was defined as a 25(OH)D below 20 ng/ml (50 nmol/l), and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (52.5–72.5 nmol/l). Bone mineral density (BMD) was studied by X-ray densitometry 'Prodigy'.
Results
Only 6.1% of people had normal level of vitamin D, vitamin D deficiency was recorded in 81.4% patients. In 16.8% the amount of 25(OH)D was below the minimal level that can machine determine. Significantly higher level of 25(OH)D recorded in summer months, and the lowest – in the winter-spring season. Secondary hyperparathyroidism diagnosed in 11.6% patients. It was found the negative correlation between the levels of 25(OH)D and parathyroid hormone ($r=-0.16$, $P=0.0001$). It was shown that only people with vitamin D deficiency have significant negative correlations between the level of parathyroid hormone and BMD at the neck and proximal femur ($r=-0.14$, $P=0.005$ and $r=-0.13$, $P=0.04$ respectively), upper and lower extremities ($r=-0.11$, $P=0.02$) and UD forearm ($r=-0.11$, $P=0.03$).
Conclusion
Deficiency of vitamin D leads the increasing of the level of parathyroid hormone, which decreasing BMD, mainly in areas of the skeleton composed of compact bone tissue.
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P158

Comparative study of bone metabolic parameters and vitamin D levels of fertile aged women, suffering from different bone metabolism altering diseases

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Introduction
Polycystic ovary syndrome (PCOS), lactase deficiency, nephrolithisasis and thyroid diseases are considered endemic. The aim of our study was to evaluate which of the above-mentioned diseases affect the most the bone metabolic parameters, bone mineral density (BMD) and the prevalence of bone fractures in fertile aged women.
Materials and methods
A total of 92 fertile aged women (32.6 ± 5.2 years) were divided into four groups (23 in each) according to their diseases (PCOS or lactase deficiency or nephrolithiasis or treated hypothyroidism). BMD was measured by dual-energy X-ray absorptiometry (DEXA). Laboratory analysis included routine and also calcium metabolism: 25-hydroxyvitamin D (25-OHD_3), parathyroid hormone (PTH), β-CrossLaps, 25-OH D_3-vitamin D, osteocalcin (OC). The prevalence of bone fractures were also recorded. Statistical analysis was performed by ANOVA, with *post-hoc* Bonferroni's correction (Satistica Software 9.0).

Results
After comparing the results of the groups, women with nephrolithiasis had the lowest BMD (Zsc: -0.92 ± 0.35) ($P < 0.05$) in the region of lumbar spines (L2–4), the lowest BMD (Zsc: -1.33 ± 0.43) ($P < 0.05$) in the region of the femoral neck, and lowest BMD (Zsc: $-1.1.9 \pm 0.31$) ($P < 0.05$) at the one third of distal radius among all groups. The average bone fracture rate of the 92 fertile aged women was high (40.8%), and serum 25-(OH) vitamin D_3 levels were low (27.8 ± 6.1–36.4 ± 6.4 nmol/l) comparing to the normal range.
Conclusion
The recognition and treatment (for e.g. sufficient supplementation of vitamin D) of endemic diseases in women in fertile age should become part of the osteological and fracture prevention.

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P159

Structure and frequency of clinical manifestations of primary hyperparathyroidism

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Introduction
Primary hyperparathyroidism (PHPT) is a result of overproduction of parathyroid hormone with pathologically changed parathyroid glands (PTG) and calcium-phosphorus metabolism disorders. Until now, PHPT is often detected late (in countries where there is no screening for hypercalcemia), when there are irreversible complications it manifested.
Materials and methods
One hundred and nine patients with PHPT (mean age: 52.2 ± 8.7 years, duration disease: $8.6 + 4.3$ years) were included in the retrospective study. The control group consisted of 55 age, gender matched subjects. Questionnaire survey (anamnesis data, risk factors for osteoporosis), parameters of calcium-phosphorus metabolism (PTH, Ca, Ca^2, P), bone markers (alkaline phosphatase, N-MID osteocalcin, β-CTX), sonography of the thyroid (PTG), scintigraphy PTG, bone mineral density (BMD) were examined.
Results
Among the examined patients the male-female ratio was 1:15 (7:102). Premenopausal women represented 28.4% (29) of the examined patients; postmenopausal women -71% (73). Mild PHPT was revealed in 23% patients, symptomatic PHPT – in 77%. Visceral form of disease was diagnosed in 21% patients, bone disorders – in 21% surveyed, mixed forms – in 35%. Low bone mineral density was detected in 75.9% (34.5%, osteoporosis; 41.4%, osteopenia) patients with PHPT; in the control group: 29.2 and 16.7% respectively. Clinical manifestations of symptomatic PHPT were: urolithiasis in 21% patients, cholelithiasis, 5.6%; a stomach ulcer and duodenal ulcer, 6.7%; fragility fractures had 11% of the examined patients.
Conclusion
The results of the retrospective study suggest the late diagnosis of PHPT, mostly the manifest forms associated with complications of PHPT and the low diagnostic rate of mild forms of PHPT, which necessitates the introduction of screening for hypercalcemia.

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P160

A case report: hypoparathyroidism, nephrocalcinosis, and replacement therapy

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Introduction
There is a risk of hypercalcemia, nephrolithiasis, nephrocalsinosis, and renal failure in the treatment of hypoparathyroidism. We present a case who has an improvement of this complication after changing large doses of vitamin D to physiological doses of $1,25(OH)_2D$ itself.
Case report
A 50 years old man was admitted to hospital. He had idiopathic hypoparatyhroidism from the age of 24. He was using oral $1,25(OH)_2D$ 0.25 μg, 1333 IU Vit D, and 2500 mg calcium carbonate and also 880 IU Vit D_3/day.
Physical examination was normal. Laboratory tests were revealed the serum creatinine as 1.3 mg/dl but serum calcium was 7.6 mg/dl and serum phosphorus was 5 mg/dl. Renal US confirmed medullary nephrocalcinosis.
The therapy was changed to $1,25(OH)_2D$ 0.25 μg 2×1/day and and 2500 mg calcium carbonate and also 880 IU Vit D_3/day.We followed serum calcium and phosphorus for monthly, serum creatinine for every 3 month and renal USG for every 6 month.
After 6 months of therapy, serum calcium was 8.8 mg/dl, serum phosphorus was 4.1 mg/dl, and serum creatinine was 1.3 but surprisingly renal US showed an important improvement in sonographic appearance of medullary calcinosis.
Conclusion
Some literature suggests the use of injectable PTH(1–34) for this complication. However, PTH is not approved by FDA for use in hypoparathyroidism in USA because of the unknown risk of osteosarcoma. In Turkey we can only use as a drug except of indication with the permission of Health Ministry.
In this case; changing the therapy to physiological doses of $1,25(OH)_2D$ and adding elementary calcium a dose for maintaining serum calcium level near the lower limit of normal reference values a degree of some improvement was provided.

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P161

Abstract withdrawn.

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P162

The prognostic value of peripheral neuropathy for the mortality and its relation to secondary hyperparathyreoidism: a 10-month follow up of 97 patients with end-stage renal disease

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Introduction and objectives
The aim of this study was to evaluate the risk of death in relation to hemodialysis.
Material and methods
We performed a 10-month follow up study of hemodialysed patients attending our Center. A total of 97 patients were evaluated: 37 (38.1%) nephroangiosclerosis, 28 (28.9%) diabetic nephropathy, 18 (18.6%), and glomerulonephritis 14 (14.4%) other causes. Somatic sensory neuropathy (PN) was documented using the Neuropathy Disability Score (NDS), vibration perception threshold (VPT) was measured by semiquantitative tuning fork C128 (grades 0–8) and AR (ankle jerk reflex) were recorded.
Results
Thirteen patients died (13.4%), 12 from the first two groups, none of the third group. Patients who died of other differed by: age (64.2 ± 11.2 vs 55 ± 12.9 years, P 0.02), CRP (13.7 ± 10.5 vs 7.2 ± 8.8 mg/l, P 0.03), BMI high (32.8 ± 6.5 vs 29.3 ± 5.9, P 0.06), AR (3.5 ± 0.9 vs 2.3 ± 1.8, P 0.02), NDS (5.8 ± 2.7 vs 3.8 ± 3.2, P 0.03), albumin level (34.7 ± 5.1 vs 38 ± 2.9 g/l, P 0.06), duration of dialisys (2.3 ± 2.3 vs 5.2 ± 4.9 years, P 0.04), and VPT (3 ± 3.2 vs 5.1 ± 2.5, P 0.001). There were no differences in parathormon (PTH) between groups (180.9 ± 264 vs 318 ± 498 pg/ml, P 0.42). Simple regression analysis of NDS of the whole 97 patients: HDL chol (r +0.23, P 0.04), Hb (r −0.20, P 0.05), BG (blood glucose; r +0.37, P 0.0003), PTH (r −0.21, P 0.047), creatinine (r −0.30, P 0.003), duration of dialisys (r −0.34, P 0,000), ABPI (ankle brachial pressure index, r +0.20, P 0.02). In multiple regression model as a significant deterioration of neuropathy (NDS) remains only: body height (P 0.005), blood glucose (P 0.08) and PTH (P 0.04).

Conclusions
This study showed that PN was a prognostic factor for death in dialysed patients and its failure was associated with elevated BG and lower PTH. So, the treatment of secondary hyperparathyreoidism in hemodialysed patient is not indicated in at least those patient with developed peripheral neuropathy.

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P163

Postsurgical hypoparathyroidism with 'normal' PTH

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Introduction
Hypoparathyroidism is characterized by hypocalcemia and low or inappropriately normal levels of PTH. The most common cause of hypoparathyroidism is iatrogenic due to anterior neck surgery. In recent years it was documented that vitamin D insufficiency is widespread.
Aim
To investigate the cause of hypocalcemia in thyroidectomized patients with PTH within the reference range.
Methods
Retrospective review of clinical records of patients who underwent thyroid surgery with hypocalcemia and PTH within reference values. PTH was measured by chemiluminescent immunoassay (Immulite 2000) with reference values between 11 and 65 pg/ml and 25-OH-vitamin D was dosed by electrochemiluminescence (Cobas e411) with reference values between 30 and 100 ng/ml.
Results
We evaluated 16 patients (88% female) with a mean age ($\pm$S.D.) of 42 ± 14 years at the time of surgery. On postoperative evaluations, all of them had 'normal' PTH values with hypocalcemia, in which 11 needed calcium and/or calcitriol. Hypovitaminosis D was documented in 13 of 14 patients (93%) in which this parameter was assessed, of whom three had vitamin D <10 ng/ml (deficiency) and ten had vitamin D between 10–30 ng/ml (insufficiency). In two patients vitamin D was not dosed. One patient had sufficient vitamin D (>30 ng/ml).
Discussion/conclusion
A normal PTH value does not exclude the presence of postsurgical hypoparathyroidism. Lack of vitamin D in patients with impaired parathyroid reserve may explain the hypocalcemia. These situations could probably benefit from treatment with colecalciferol.

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P164

Evaluation of concentration 25OH vitamin D_3 selected patient population: preliminary report

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Introduction
Vitamin D in the human body plays a very important role.
Aim
The aim of the study was identify the differences in concentration of 25OH vitamin D_3 in blood and compared with anthropometrics and biochemicals results of researched group of people.
Materials and methods
To research were included 212 patients hospitalized on Endocrinology Department of Hospital in Poland in 2012. Patients were divided into three groups adequately to the division created by Erik Erikson. As a correct values of 25OH vitamin D_3 was adopted level above 30 ng/dl, as deficiency values 12–30 ng/dl, severe deficiency below <12 ng/dl. Obtained data were collected and analyzed in STATISTICA 10 program (statistical significance $\alpha = 0.05$).
Results
The study included people aged 21–82 years. Among 74% of researched people were observed deficiency of 25OH vitamin D_3, 15% of this group has severe deficiency. Significant statistical differences were observed between BMI in first and second group ($P = 0.041$); waist measurement value (WC) ($P = 0.001$), WHR value ($P = 0.00002$), the value of the WHtR ($P = 0.00021$) in the first group and the second as well as the first and third.The differences were observed between concentration of total cholesterol level ($P = 0.005$), cholesterol LDL fraction ($P = 0.015$) and level of glycemic ($P = 0.036$) in first and second group.
Conclusions
Obtained results in research show that in age range 21–65 with age there is an increase of values: BMI, WHR, WHtR, WC and increase concentration the level of total cholesterol, cholesterol LDL and glycemic levels.

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P165

Evaluation of concentration 25OH vitamin D_3 in the population over 65 years old: preliminary report

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Introduction
Old age is defined as late adulthood and is an important part of human's life. This is the time of life, when exist the real risk of 25OH vitamin D_3 deficiency.
Aim
The aim of the study was find out the connections between concentration of 25OH vitamin D_3 in blood and anthropometrics and biochemical results depending on diseases in persons above 65 years.
Materials and methods
The research was conducted among patients hospitalized on Endocrinology Department of Hospital in Poland in 2012. Among research group chosen 50 persons which met the inclusion criteria: as age above 65 years. As a correct value of concentration of 25OH vitamin D_3 in blood was adopted the level above 30 ng/dl, deficiency level 12–30 ng/dl, for the severe deficiency value below 12 ng/dl. The results were collected and analyzed in STATISTICA 10 program (statistical significance $\alpha = 0.05$).
Results
The research were conducted among persons in age 65–82 years. Mean concentrations ($\pm$S.D.) 25OH vitamin D_3 was 20.8 ± 7.2 ng/dl. Among 80% of researched group was observed deficiency of concentration of 25OH vitamin D_3, in this group 10% of researched persons had a severe deficiency Significant statistical differences were observed between concentration of 25OH vitamin D_3 and waist circumference ($P = 0.022$). It was also a statistically significant correlation between serum 25OH vitamin D_3 and WHtR index ($P = 0.018$).
Conclusions
The results indicated that the lower concentrations of 25OH vitamin D_3 were accompanied with higher value of index WHtR and higher values of waist circumference.

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P166

Cardio-metabolic risk factors in subjects with Vit D deficiency

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Introduction
Vitamin D (Vit D) is known for its action on bone metabolism. But recently more ubiquitous actions have been discovered. Vit D deficit is now considered as a world health problem because of it frequency and its consequences. Vit D deficiency seems to be involved in diabetes mellitus, and in autoimmune, neoplastic and cardio-metabolic diseases. The aim of our study was to analyze the incidence of cardio-metabolic diseases in a group of patients with vitamin D deficiency.
Subjects and methods
This retrospective study analyzed 23 patients with vitamin D deficiency (25OH vit D levels <30 ng/ml) diagnosed during the exploration of normocalcemic hyperparathyroidism.
Results
Mean age was equal to 57.5 ± 11 years. The mean BMI $= 30.8 \pm 7.21$ kg/m^2. A BMI ≥ 25 kg/m^2 was found in 87% of our patients (56% were obese and 31% were overweighed). Systemic high blood pressure was observed in 34.7%. 21.7% were dyslipidemic and 21.4% had glucose metabolism disorders.
Conclusion
In this study, although our subjects are relatively young, they are at high risk for cardio-metabolic diseases especially for obesity. But, as obesity interact on vitamin D deficiency; it is difficult to say which is the most deleterious for cardiovascular diseases. So, in future, prospective and large series with multivariate analyses are needed to confirm our results.

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P167

Hyperparthyroidism: are male forms more or less aggressive?

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Introduction
Hyperparathyroidism (HPT) is a common disease prevailing in females (F). Male (M) forms are rare and their impact on the body is less studied than in females. Our aim is to see if male HPT is more or less aggressive than the female one.
Subjects and methods
It is a retrospective study that took in account 62 HPT (46F, 16M) over a period of 7 years (2005–2012). All were questioned, examined, and had routine analyses, biological assessment based on PTH, blood and urinary calcium (ca), and phosphorous (P). Plain X-ray, ultrasounds, intravenous urography, and MIBI scintigraphy were done too. For statistical analyses Student's t-test and χ^2 were performed for comparison. The difference was considered as significant if $P<0.05$.
Results
Mean age was equal to 53 for F and 46 for M ($P=0.10$). Women consulted for bone problems in 47 vs 12.5% for men ($P=0.01$). Kidney abnormalities were observed in 21% F and 31% M ($P=0.66$). Mean ca $=$ 101 vs 111 mg/ml, $P=0.21$; phosphorous $=$ 29.8 vs 37.5 mg/l, $P=0.57$; PTH $=$ 291 vs 320 pg/ml, $P=0.59$. Bone problems were evident in 50% F and 58% M, but $P=0.54$. Kidney impact was observed in 21.7% F and 31% M, $P=0$ .37. Primary HPT $=$ 55% in F, 68% in M, but $P=0.31$. Secondary HPT was found in 45% F vs 31.25% in M, but $P=0.31$. Parathyroid adenomas were located in the inferior glands (50% right, 50% left) in F vs indifferent locations for M.
Conclusion
In this study where male HPT are less frequent than women's, we observed that women suffer more than men from their bones, probably because of the additional menopause and vitamin D deficiency, but statistical analysis did not show any objective difference between men and women forms.

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P168

A clinical case of complicated hyperparathyroidismwith diagnosis delay due to vitamin D deficiency

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We present the case of a 75-year-old woman with a 10-year history of nephrolithiasis, for which she endured several sessions of extracorporeal shockwave lithotripsy (ESWL). She was directed to the Endocrinology unit for further investigations, due to persistent albeit minor increased values of blood calcium (10.48 mg/dl, normal 8.4–10.2 mg/dl). Here, the clinical supposition of primary hyperparathyroidism was confirmed. The tests revealed a mild hypercalcemia with hypophosphatemia (2.25 mg/dl, normal 2.5–4.7 mg/dl) and hypercalciuria (408 mg/24 h normal values 100–300 mg/24 h). Although at advanced age and never been treated for osteoporosis, she had three pathological fractures and very low bone mineral density at all locations, radius included. These antecedents increased the supposition of supplementary metabolic disorder, other than postmenopausis and/or senile status.
PTH levels were abnormally increased (295 pg/ml, normal levels 11–67 pg/ml) and 25HO-D vitamin level was low (10.59 ng/ml, normal >30 ng/ml).
A hypoechogenic well delimited nodule was detected behind the lower pole of the left thyroid lobe. This image overlapped on a region of increased uptake by scintigraphic with sesta-MIBI, confirming the location of a parathyroid adenoma. We recommend the patient minimal invasive surgery and bone protection with bisfosphonates, after which we estimate a favorable evolution.
Primary hyperparathyroidism is a common endocrinologycal disease usually. Its presence is usually explained by hypercalcemia. Delay was due to masked hypercalcemia by vitamin D deficiency.

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P169

Impact of vitamin deficiency and oF GH–IGF1 on cardiovascular risk in hypopituitaric patients

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To determine a correlation between Vitamin D, GH, IGF1 and cardiovascular risk we enrolled. 41 GHD patients (22 M, 19 F, age 18–84 years) and 41 controls. In all we determined: anthropometric parameters, blood pressure (BP), lipid and glucose profile, PTH, 25-OH-vitamin D, GH peak after GHRH $+$ ARG, IGF1. Metabolic syndrome (MS) was evaluated by the IDF criteria.
The vitamin D were lower in patients than in controls (21.3 ± 12.3 vs 28.2 ± 9.4, $P=0.006$). A deficiency of vitamin D (<20 ng/ml) was found in 51 vs 14.6% ($P=0.000$), insufficiency (20–30 ng/ml) in 26.8 vs 41.4% ($P=0.27$) and normal vitamin D (>30 ng/dl) in 21.9 vs 43.9% ($P=0.06$) respectively in patients and controls. The prevalence of dyslipidemia was 51.2 vs 12.1% ($P=0.09$), DM was 7.3 vs 17% ($P=0.292$) of hypertension was 44 vs 22% ($P=0.06$), MS was 17 vs 14.6% ($P=0.957$) respectively in patients and controls. In both groups there was a significant correlation between IGF1, age, vitamin D and SBP. At the multiple regression, the greater predictor of high values of SBP were IGF1 ($t=-2.69$, $P=0.011$, $t=-0.18$, $P=0.018$ respectively). At logistic regression only in patients we found a significant association between IGF1 and vitamin D deficiency and dyslipidemia and hypertension, but not with DM. The MS was significantly associated only with vitamin D. At the multiple logistic regression, vitamin D was associated with dyslipidemia and hypertension.
In GHD hypopituitaric patients the vitamin D is more associated with cardiovascular risk factors. Thus, it can be assumed that the vitamin D deficiency may represent an additional risk factor to the already known effects of hypopituitarism for cardiovascular diseases.

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P170

Primary hyperparathyroidism in patients with urolithiasis: prevalence and predictors

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Objective
To know the prevalence of primary hyperparathyroidism (PHPT) in urolithiasis and predictors of PHPT in urolithiasis.
Methods
This cross sectional study was conducted between July 2005 and July 2012 in department of endocrinology and urology at our hospital. In this study a total of 165 consecutive patients with urolithiasis with radioopaque stones were evaluated for clinical and biochemical profile. Stones retrieved were analyzed for type of stone using infrared spectroscopy.
Results
Out of these 165 patients, 123 were males, 42 females, with a mean age of 34.5 ± 12.1 years. Eleven patients (7%) had histopathologically proven PHPT. Mean age of these patients was 45.6 ± 12.4 years with male: female ratio of 4:7. Prevalence of bone pains, backache, fracture, weakness, fatigability, joint pain, and myopathy were more common in PHPT group. Three (35%) patients in PHPT and 3 (2%) in non PHPT group had nephrocalcinosis ($P<0.0001$). Simultaneous renal and ureteric stones were significantly more common in PHPT patients ($P<0.0001$). Calcific pancreatitis was found in 2 (18.1%) patients with PHPT as compared to 1 (0.6%) in non PHPT group (P value 0.0001).
Mean serum calcium in PHPT group was 12.3 ± 0.3, 9.2 ± 0.03 g/dl in non PHPT group ($P<0.0001$), Alkaline phosphatase was 312.5 ± 73.9 vs 114.3 ± 2.6 IU/l ($P<0.0001$) and phosphate was 3.3 ± 0.3 vs 3.7 ± 0.5 g/dl (P value = 0.4), iPTH levels were 398.9 ± 132.4 vs 49.3 ± 3.1 pg/ml ($P<0.0001$) respectively. Calcium oxalates were the most common type of stones in either of the groups (85.3 vs 69.1%) respectively.
Discussion
The prevalence of PHPT in our patient with urolithiasis is 7%. Urolithiasis is more common in males but prevalence of PHPT is more common in females with urolithiasis. Urolithiasis patients with PTPH are older with additional symptomatology. Serum calcium, alkaline phosphate, parathyroid hormone levels were predictors of PHPT. Nephrocalcinosis, concomitant ureteric and renal stones and calcific pancreatitis were predictors of PHPT in urolithiasis patients.
Conclusion
PHPT should be considered as an etiologic factor in urolithiasis.

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P171

Could PHPT be diagnosed at early stages even more frequently?

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Primary hyperthyroidism (PHPT) is one of the diseases which are usually diagnosed in asymptomatic period nowadays. Even though considerable success was achieved, in some cases PHPT is not found out before complications were developed. A prospective study focuses on diagnostics and therapy of PHPT has been in progress at two tertiary centers of endocrinology in the Czech Republic since January 1st, 2007, with early diagnosis of PHPT being one of its aims. In a 6-year period, there were 217 patients with PHPT. There were 29 hypercalcemic patients. Their serum calcium level exceeded 2.60 mmol/l. 188 normocalcemic patients had serum calcium level 2.60 mmol/l or less at the first examination. Intermittent or constant hypercalcemia developed in 28 patients who were originally normocalcemic. Four years was the longest period during which constant hypercalcemia developed in an originally normocalcemic patients. Set of symptoms being characteristic of PHPT was present only in ten patients when they were included into the study group, later on some clinical features appeared in other 14 patients. The most common complaints were fatigue, gastrointestinal signs, muscular and bone pain and psychiatric problems, especially depression. There was stated a frequent coincidence of PHPT and disorders of pituitary gland. Therefore it was started with parathyroid hormone serum level examination of patients treated at the centre of endocrinology in the time of study. Normocalcemic PHPT was found out at 107 endocrine patients.
Conclusions
Serum parathyroid hormone level examination in asymptomatic patients seems to be the most important tool of PHPT early detection. A frequent coincidence of PHPT and other endocrine disorders was found out, this finding needs to be verified. Permanent hypercalcemia can be developed in a normocalcemic patient with PHPT even after a 4-year period.

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P171.1

Relation between carotid intima media thickness and vitamin D in hypertension

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Introduction
Hypertension is associated with increased risk of vascular disease. There is possible relation between low vitamin D levels and subclinical atherosclerosis defined as increased carotid intima media thickness (CIMT). Vitamin D deficiency is defined as levels below 20 ng/ml. In this study we aimed to find out the relation between 25 hydroxy D vitamin level and CIMT and the presence of atherosclerotic plaques.
Methods
A total number of 163 patients (15 male and 148 female subjects) with essential hypertension aged between 30 and 76 were included to the study. The patients were already on hypertensive medication. CIMT was measured and the presence of plaque was evaluated by real time B mode ultrasonography. Serum parathormone and 25 OH vit D levels were measured using ELISA. None of the patients were on steroid medication. All parametric variables were evaluated with Student's t-test using SPSS 10.0. The relation between 25 OH vitamin D level and MCIMT was evaluated with Pearson correlation analysis. χ^2 test was done for nonparametric variables. P below 0.05 was considered statistically significant.
Results
Patients with plaques were older than those without plaques ($P=0.0001$) and had longer duration of hypertension ($P=0.008$) and higher TSH level ($P=0.06$). Eighty had plaques. Seventy three patients had severe vitamin D deficiency defined as level below 10 ng/ml and 64 had insufficiency, (10–20 ng/ml). Vitamin D levels were similar in both groups ($P=0.44$) but those with plaques had higher MCIMT ($P=0.0001$). MCIMT was positively correlated with age ($r=0.399$, $P=0.0001$), fasting glucose ($r=0.165$, $P=0.036$), and HbA1c ($r=0.384$, $P=0.002$). There was no correlation between Vitamin D level ($P=0.75$) and LDL ($P=0.581$).
Conclusion
We did not find any relation between vitamin D level and MCIMT in hypertensive subjects. The lack of significance may be due to high prevalence of vitamin D deficiency. There was no correlation between vitamin D level ($P=0.75$) and LDL ($P=0.581$).

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Cardiovascular Endocrinology & Lipid Metabolism

P172

Lipid levels in acromegaly

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Acromegaly is known to be associated with increased cardiovascular risk. Additionally, acromegaly is known to be associated with disordered carbohydrate metabolism. Lipid levels in acromegaly have not been extensively studied. The aim was to study lipid levels in acromegaly.
Methods
In 32 patients with newly diagnosed acromegaly lipid levels were studied. In particular total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels were measured. All patients had a pituitary adenoma. All of them had increased IGF1 levels. The measurements were also performed in 32 control subjects matched for age and sex.

Results
Total cholesterol was 220.59±8.24 mg/dl (mean±S.E.M.) in patients with acromegaly as opposed to 198.55±4.85 mg/dl in the control subjects ($p < 0.001$, Student's *t*-test). HDL cholesterol levels were 52.96±2.89 mg/dl in patients with acromegaly as opposed to 58.44±3.62 mg/dl in the control group ($P < 0.001$). LDL cholesterol was 151.70±13.77 mg/dl in the acromegalic patients as opposed to 114.06±5.31 mg/dl in the control group ($P < 0.001$). Triglyceride levels were 140.34±14.79 mg/dl in patients with acromegaly as opposed to 133.50±14.27 mg/dl in the control group ($P < 0.001$). Thus, total cholesterol, LDL cholesterol and triglyceride levels were increased in patients with acromegaly as opposed to the control group, while HDL cholesterol was decreased.
Conclusions
It appears that acromegaly is associated with a proatherogenic lipid profile, which may contribute to the increased cardiovascular risk associated with the disease.

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P173

Testosterone stimulates cholesterol metabolism and efflux from human macrophages via liver X receptor

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Low testosterone is associated with an increased prevalence of cardiovascular (CV) disease. Testosterone replacement improves several CV risk factors including lowering cholesterol and may protect against atherogenesis. Macrophage liver X receptor α (LXRα) stimulates cholesterol efflux which is a therapeutic target for the treatment of atherosclerosis. It was therefore proposed that the anti-atherogenic effect of testosterone may be mediated via LXRα. THP-1 macrophages were used, as they express the androgen receptor (AR) and are therefore responsive to testosterone. Cells were treated with testosterone, either alone or in combination with Flutamide (AR inhibitor) or LXR antagonist and gene expression between control and treated cells was assessed by qPCR. A fluorescent cholesterol analogue was used to observe the effect of testosterone on cholesterol efflux. Protein localisation was observed by immunofluorescence. Testosterone significantly increased the expression of LXRα and of LXRα-target genes encoding proteins involved in cholesterol efflux and metabolism, including *ABCA1* (ATP-binding cassette transporter A1), *APOE* (apolipoprotein E), *FAS* (fatty acid synthase), and *SREBP1c* (sterol regulatory element-binding protein 1c). Blocking LXRα activity inhibited the effect of testosterone, demonstrating testosterone increases *ABCA1, APOE, FAS*, and *SREBP1c* expression by activating LXRα. Testosterone was shown to act via its AR to activate LXRα, as blocking the AR inhibited the effect of testosterone on LXRα and its downstream targets. Testosterone increased the rate of cholesterol efflux from macrophages; a high level of ABCA1 protein at the cell membrane in testosterone-treated cells suggests this may be due to an increase in intracellular cholesterol transport. We provide evidence that testosterone activates LXRα and acts through this nuclear receptor to control the expression of LXR-target genes to stimulate cholesterol efflux and metabolism.

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Testosterone differentially regulates liver X receptor expression and targets of lipid and glucose metabolism in liver, muscle and adipose tissue of the testicular feminised mouse

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Testosterone deficiency increases risk for cardiovascular disease (CVD) and type 2 diabetes. Testosterone replacement (TRT) improves insulin resistance, glycaemic control and cholesterol in hypogonadal men. Liver X receptor (LXR) is a nuclear receptor which regulates lipid and glucose metabolism. LXR agonists protect against atherosclerosis but cause hepatic steatosis. We have previously shown that TRT protects against hepatic steatosis and atherosclerosis in high-fat diet-fed testicular feminised (Tfm) mice, which exhibit non-functional androgen receptors (AR) and low circulating testosterone levels. This study investigated LXR expression and key downstream targets involved in lipid and glucose homeostasis in liver, muscle and adipose tissue of Tfm mice.
High-cholesterol diet-fed Tfm mice received either TRT or placebo and were compared to wild-type littermates. Liver, muscle, visceral adipose and subcutaneous adipose tissue mRNA and protein were analysed by qPCR and western blotting for LXR expression and downstream targets involved in lipid metabolism (acetyl coA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL), apolipoprotein E (ApoE), and ATP-binding cassette transporters (ABC-A1, ABC-G5)), glucose control (glucose transporter 4 (GLUT4), hexokinases (HK2, HK4), and phosphofructokinase (PFK)) and master regulators of lipid and glucose metabolism (peroxisome proliferator-activated receptors (PPARα; PPARγ) and sterol regulatory element-binding proteins (Srebp1, Srepb2)).
LXR was down-regulated in liver and subcutaneous adipose of Tfm mice compared to wild-type with TRT increasing LXR expression. LXR was not altered in muscle or visceral adipose. Downstream targets of LXR were altered in the liver (↑FAS, ↑ACC, ↓ApoE, ↓ABC-A1, ↓HK4, and ↓PFK) muscle (↓HK2, ↓GLUT4, and ↓PFK) and subcutaneous adipose (↓LPL, ↓ABC-A1, ↓ApoE, ↓HK2, ↓GLUT4, ↓Srebp-1, and ↓Srebp-2). All other targets in alternate tissues were not affected. TRT returned hepatic FAS, ACC, ApoE, ABC-A1, HK4; muscle GLUT4 and subcutaneous ApoE, HK2, Srebp-1, Srebp-2 to wild-type levels.
Testosterone may act through LXR to influence carbohydrate and lipid metabolism as a mechanism to improve insulin resistance and reducing cardiovascular risk.

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P175

Oxytocin, a new regulator of cardiomyocyte hypertrophy

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We have uncovered the cardioprotective functional oxytocin (OT) system in the rat and human heart. In rat model of heart infarct, OT treatment significantly reduced infarct size, decreased cardiomyocytes (CM) diameter, increased expression of atrial natriuretic peptide (ANP) and improved parameters of heart function. Consequently, in this report we investigated hypothesis that OT-related mechanism of ANP synthesis or release, plays a role in the control of CM hypertrophy.
The experiments were carried out in culture of newborn and adult rat CM in which hypertrophy was stimulated by endothelin-1 (ET-1, 100 nM). After 24-h CM hypertrophy was assessed by increased protein synthesis (30% as determined by [^{35}S]-methionine incorporation) and increased cell surface area (increase by 70%). OT (100 nM) treatment completely abolished hypertrophic effect of ET-1 in CM and reduced hypertrophy induced by other GqPCR agonists, such as the angiotensin II and phenylephrine. OT dose-dependently increased ANP release from the CM, ANP accumulation in the cell perinuclear region and increased the intracellular cGMP concentrations in newborn rat CM. However, the ANP receptor blockade by anantin did not completely inhibited cGMP enhancement in CMs exposed to OT suggesting also contribution of NO. Indeed, OT-mediated cGMP production in CM were reduced by l-NAME, a non specific inhibitor of NO synthases, partially reduced by 1400 W, an inhibitor of inducible NOS, and ODQ, an inhibitor of NO-sensitive guanylyl cyclases. STO-609 and compound C inhibition of anti-hypertrophic OT effects in CM indicated also the contribution of calcium–calmodulin kinase kinase and AMP-activated protein kinase pathways. Western-blot demonstrated that OT stimulates phoshorylation of Akt/PI3K pathway. Moreover, OT treatment normalized Akt phosphorylation reduced by ET-1, prevented abundant accumulation of ANP in hypertrophic cells and blocked ET-1-mediated translocation of transcription factor, NFAT, into the cell nuclei.
In conclusion, anti-hypertrophic OT effect in CM involves Akt/PI3K and AMPK signaling cascades. cGMP/protein kinase G mediate OT-induced anti-hypertrophic response with contribution of ANP and NO.

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P176

Long QT interval in Turner syndrome: a high prevalence of LQTS gene mutations

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Objective
QT interval prolongation of unknown aetiology is common in Turner syndrome (TS). This study set out to explore the presence of known pathogenic long QT (LQT) mutations in TS and to examine the corrected QT interval (QTc) over time and relate the findings to the TS phenotype.
Methods
Adult females with TS ($n=102$) were examined thrice with a mean follow-up of 4.7 ± 0.5 years, and 68 age-matched healthy controls were examined once. QTc was measured by one blinded reader (intra-reader variability: 0.7%), and adjusted for influence of heart rate by Bazett's (bQTc) and Hodges's formula (hQTc). The prevalence of mutations in genes related to Long QT syndrome (LQTS) was determined in females with TS and a QTc >432.0 ms. Echocardiographic assessment of aortic valve morphology, 24-h blood pressures and blood samples were done.
Results
The mean hQTc in females with TS (414.0 ± 25.5 ms) compared to controls (390.4 ± 17.8 ms) was prolonged ($P<0.001$) and did not change over time (416.9 ± 22.6 vs 415.6 ± 25.5 ms; $P=0.4$). 45,X karyotype was associated with increased hQTc prolongation compared to other TS karyotypes (418.2 ± 24.8 vs 407.6 ± 25.5 ms; $P=0.03$). In females with TS and a bQTc >432 ms, seven had mutations in major LQTS-genes (SCN5A and KCNH2) and one in a minor LQTS-gene (KCNE2).
Conclusion
The prevalence of mutations in major LQTS genes was strikingly high for females with TS and the longest QTc interval.

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P177

Osteoprotegerin and RANKL levels in instable coronary artery disease

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Introduction
Osteoprotegerin (OPG) and receptor activator for nuclear factor-κB ligand (RANKL) are cytokines that increase in cardiovascular diseases. Serum OPG levels are high in vascular calcifications, while RANKL is expressed by atherosclerotic lesions. The aim of the study was to assess the OPG and RANKL concentrations in patients with stable coronary artery disease.
Material and methods
The study included 38 patients diagnosed with ischemic coronary disease (16 men and 22 women, mean age 54.3 ± 7.2 years) and 34 control subjects, sex and age-matched. Analyses for serum cholesterol, high and low-density lipoprotein (HDL and LDL) cholesterol, triglycerides, and glucose were performed by enzymatic methods. High-sensitivity C-reactive protein (hsCRP), OPG and RANKL were measured by ELISA.
Results
Serum OPG levels were significantly higher in study group than in controls (3.52 ± 0.75 vs 1.19 ± 0.35 pmol/l, $P<0.0001$). RANKL (pmol/l) to OPG (pmol/l) ratio was significantly higher in study cases than in controls (0.48 ± 0.12 vs 0.30 ± 0.12, $P<0.0001$). OPG values did not correlate with age, cholesterol, HDL-cholesterol, triglycerides and hsCRP. A significant correlation was found between RANKL and OPG: $r=0.530$, $r^2=0.281$, $P=0.003$. RANKL/OPG correlated positively with total cholesterol ($r=0.509$, $r^2=0.295$, $P=0.004$) and hsCRP values ($r=0.453$, $r^2=0.205$, $P=0.01$), but it didn't correlate with age, triglycerides and glucose. There were not significant differences between men and wome regarding: OPG, RANKL/OPG, hsCRP levels and lipid profile. Receiver-operating curve analysis showed that at a cutoff point of 0.381, RANKL/OPG presented a sensitivity of 86.2%, but a lower specificity (76.6%). At a cutoff point of 2.52 pmol/l, OPG showed a better sensitivity (91.2%) and specificity (89.1%). At a cutoff point of 2.95 mg/l, hsCRP presented a moderate sensitivity (79.3%), but a good specificity (93.1%).
Conclusion
Our study confirms that OPG and RANKL/OPG values are higher in ischemic patients, correlated with other inflammatory markers (hsCRP). RANKL/OPG could represent a good assessment and prediction parameter of future cardiac events.

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P178

Intestinal cholesterol-transport gene expression is associated with abnormalities in post-prandial endothelial function and carotid intima-media thickness independent of insulin resistance

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Introduction
Intestinal cholesterol absorption is known to be important in post-prandial lipid levels, and implicated in the development of vascular disease. This study evaluated the effect of intestinal gene expression on markers of early vascular disease in a cohort of diabetic and non-diabetic subjects.
Methods
Subjects were identified from routine upper GI lists and enrolled as per local ethics approval. Intestinal gene expression was measured on duodenal biopsy samples, using rtPCR to measure mRNA levels of Niemann-Pick C1-L1, ABCG5, ABCG8, ABCA1, microsomal tissue transport protein (MTTP), and sterol regulatory element-binding protein types 1 and 2. Following a standard mixed meal, serum lipid profiles were measured and insulin resistance was measured via HOMA-IR. Post-prandial endothelial function was assessed via flow-mediated dilatation (FMD) of the brachial artery, and carotid IMT measured using B-mode ultrasonography. Statistical analysis utilised SPSS (Ver 10.1).
Results
One hundred patients were analysed. Intestinal gene expression was associated with alterations in IMT and post-prandial FMD as demonstrated in Table 1, corrected for age, BMI and HOMA-IR.
Fasting and post-prandial lipid levels were not associated with either vascular markers or intestinal gene expression. Gene expression was not affected by smoking status, diabetic staus or gender.

Table 1

	NPC1L1	ABCG5	ABCG8	MTTP	ABCA1	SREBP1	SREBP2
IMT	0.147[a]	0.349[†]	0.324[†]	0.274[*]	0.229[*]	0.076[a]	0.296[†]
PP FMD	−0.245[*]	−0.197[a]	−0.246[*]	−0.253[*]	−0.358[†]	−0.363[†]	−0.287[†]

[*]$P<0.05$, [†]$P<0.01$, [a]NS. Values are for R (Pearson's correlation coefficient).

Conclusion
Intestinal gene expression shows significant association with increased IMT, a marker for early atherosclerotic change, as well as reduced post-prandial FMD, a marker of early endothelial dysfunction. This association appears to be independent of post-prandial lipid levels and glycaemic status.

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P179

Mediterranean diet modulates the effect of rs1761667 in the *CD36* gene on FFA concentration and BMI in a high cardiovascular risk population

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Introduction and objective
CD36 is a scavenger receptor that facilitates the membrane transport of long chain fatty acids into muscle and adipose tissue. Polymorphisms in this gene have been related to abnormal serum fatty acids and obesity-related phenotypes with inconsistent results. Gene-diet interactions could modulate these associations. Our aim was to study whether a common functional variant (rs1761667) in this gene will associate with anthropometric variables and plasma free fatty acids (FFA) as well as the modulation by the adherence to the Mediterrranean diet (MD) in a high cardiovascular risk (CVR) population.
Material and methods
We studied 1036 subjects with high CVR participating in the PREDIMED (PREvención Dieta MEDiterránea) study, recruited in Valencia. Anthropometric data was obtained and plasma FFA were determined. Adherence to MD was evaluated through a validated questionnaire. Individuals were classified according to the score: low adherence (<9 points) or high adherence (≥ 9 points).
Results
Minor allele frequency (G) was 0.427. A recessive model was considered (A-allele carriers vs GG homozygous). Homozygous GG subjects had higher FFA concentrations than carriers of the A allele (GG: 18.9 ± 7.8 mg/dl vs AA + AG: 17.4 ± 6.7 mg/dl, $P=0.035$). Interestingly, we observed an interaction (P-int: 0.049) between rs1761667 polymorphism and adherence to the MD in determining FFA concentrations (P-int = 0.049) and BMI (P-int = 0.019). So, in subjects with low adherence to MD, the GG genotype showed higher FFA concentrations (GG: 20.3 ± 7.8 mg/dl vs AA + AG: 17.5 ± 6.4 mg/dl, $P=0.001$), and BMI (GG: 32.1 ± 5.6 kg/m^2 vs AA + AG: 31.0 ± 5.0 kg/m^2, $P=0.046$) compared with A-allele carriers. But, in subjects with high adherence to MD no significant differences were observed according to genotype in this parameters (FFA, $P=0.702$; BMI, $P=0.555$).
Conclusion
Adherence to MD modulates the effect of the CD36-rs1761667 polymorphism on FFA concentration and BMI in a high CVR population.

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P180

The effect of aerobic exercise on ectopic lipids intramyocellular, intrahepatocellular, and intracardiomyocellular lipids in physically active, healthy individuals

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Introduction
Ectopic lipids are fuel stores in non-adipose tissues (i.e. skeletal muscle (IMCL), liver (IHCL), and heart (ICCL)). It is recognized that IMCL can be depleted by physical activity and repleted by dietary fat intake indicating metabolic flexibility. In contrast, preliminary data suggested that aerobic exercise increases IHCL. Data on the acute effect of aerobic exercise on ICCL is scarce.
Increased IMCL and IHCL have consistently been related to impaired insulin action in skeletal muscles and liver, while initial studies do not show such a relationship for the heart. Hypothesis: fat mass and insulin sensitivity influence the flexibility of ectopic lipids.
Methods
Ten males, physically active subjects (age: 28.9 ± 6.4 years, mean $\pm$ S.D.; VO_{2max}: 56.3 ± 6.4 ml/kg per min, BMI: 22.75 ± 1.4 kg/m^2) were recruited. VO_{2max} was assessed by ergometry. Insulin sensitivity was calculated by HOMA-Index. Visceral and subcutaneous fat mass were separately quantified by MR-imaging. Following a standardized dietary fat load over 3 days IMCL, IHCL and ICCL were measured using MR-spectroscopy before and after a 2 h-exercise at 50–60% of VO_{2max}. Effects of exercise on IMCL, IHCL, and ICCL were probed using paired *t*-tests. Correlations between relative changes in these lipids and insulin sensitivity and visceral/subcutaneous fat mass were evaluated.
Results
A 2 h-exercise resulted in a significant decrease in IMCL ($-17 \pm 22\%$, $P=0.008$) and ICCL ($-17 \pm 14\%$, $P=0.002$) and an increase in IHCL ($42 \pm 29\%$, $P=0.004$). In this cohort no significant correlations were found between the relative changes in IMCL ICCL, IHCL, and fat mass or HOMA-index.
Conclusions
These results underscore the fact that all ectopic lipids are flexible fuel stores that are influenced by physical exercise.
In this homogenous group, physical exercise consistently decreased both ICCL and IMCL and increased IHCL.

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P181

Can hypothyroidism manifest as ischemic heart disease in elderly patients with the absence of significant coronary atherosclerosis?

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In elderly patients with symptoms of ischemic heart disease (IHD) coronary angiography often fails to reveal significant atherosclerosis. The causes of the absence of significant coronary atherosclerosis (SCA) in these patients have not been determined.
Purpose
To determine predictors of SCA absence in the elderly IHD patients.
Methods
This is a retrospective study of consecutive 10 713 patients who underwent coronary angiography at the Tyumen Cardiology Centre in 1991–2011. We selected 1483 patients with IHD and without SCA: 124 patients 65 years old and older (group I) and 1.359 patients younger than 65 years (group II).
Results
In patients of group I the incidence of hypothyroidism was higher and incidence of myocardial infarction (MI) was lower compared to group II. Echocardiographic indices of left ventricular (LV) posterior wall thickness, aortic root and left atrium in group I patients were higher. LV diastolic dysfunction was more frequent in patients of group I. According to the results of multivariate analysis LV diastolic dysfunction (OR = 2.64; 95% CI 1.21–5.77; $P=0.001$), hypothyroidism (2.35; 1.45–3.81; 0.015), increased indices of LV posterior wall thickness (1.67; 1.45–3.81; 0.019), left atrium (1.31; 1.09–2.55; 0.002), aortic root (1.26; 1.11–1.55; 0.006) and the absence of prior MI (0.34; 0.15–0.79; 0.011) were independent predictors of the SCA absence in elderly patients with IHD.
Conclusions
Hypothyroidism was strong independent predictor of the SCA absence in elderly IHD patients. Hypothyroidism as a cause of microvascular myocardial dysfunction could manifest clinically as IHD in elderly patients with the absence of SCA.

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P182

Short-term changes in serum sex steroid levels and cardiac function in healthy young men

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Introduction
Male obesity is associated with an increase in estradiol (E_2) and a decrease in testosterone (T). And, although sex steroids are associated with cardiovascular disease, direct effects on cardiac structure and function are hardly investigated in humans.
Methodology
Twenty healthy men aged 20–40 years were randomized into two groups. One group was given an aromatase inhibitor (letrozole) only, thus obtaining a high

testosterone and low E_2 (group T). The other group received an aromatase inhibitor plus an E_2 patch (dermestril), reaching a low testosterone and high E_2 (group E). Serum levels of both testosterone and E_2 remained within the normal reference range. The men underwent an echocardiography by a single cardiologist before the start of the intervention and after 7 days.

Results

Total and free E_2 serum levels were positively associated with ejection fraction ($r=0.7$, $P=0.002$ and $r=0.6$, $P=0.007$ respectively) at baseline in the whole group. In group E global circumferential strain decreased significantly from $-25.3\% \pm 3.9$ to $-19.6\% \pm 2.5$ after 1 week as compared to baseline ($P=0.01$). No significant changes in systolic function were observed in group T. Cardiac structure remained unaltered.

Conclusion

In young healthy men, an increase in E_2 and decrease in testosterone levels significantly decreased circumferential strain. The finding justifies larger studies of longer duration to discover the exact nature of the impact of changed sex steroids on cardiac function and remodelling in obesity.

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P183

Mendelian randomization suggests non-causal associations of testosterone with cardiometabolic risk factors and mortality

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Background

Prospective studies showed that low serum testosterone concentrations are associated with various cardiometabolic risk factors and mortality. But the causal nature of these associations is controversial.

Methods

We studied 1882 men aged 20–79 years with serum testosterone concentrations and genotyping data from the longitudinal population-based Study of Health in Pomerania.

Testosterone concentrations were cross-sectionally associated with cardiometabolic risk factors including anthropometric, lipid, blood pressure, and glycemic parameters; and prospectively with all-cause mortality (277 deaths, 14.7%) during the 10-year follow-up. To overcome problems of residual confounding, reverse causation, or regression dilution bias in the investigated testosterone-outcome associations, we used two-stage least square regression models with previously identified polymorphisms at the *SHBG* gene (rs12150660) and X chromosome (rs5934505) as multiple genetic instruments in an instrumental variable (IV) approach, also known as Mendelian randomisation.

Results

In standard regression analyses, testosterone was robustly associated with a wide range of cardiometabolic risk factors. In subsequent IV analyses, no such significant associations were observed. Similarly, prospective analyses showed a consistent association of low testosterone concentrations with increased all-cause mortality risk, which was not apparent in subsequent IV analyses.

Conclusion

The present Mendelian randomization analyses did not detect any evidence for causal associations of testosterone concentrations with cardiometabolic risk factors and mortality, suggesting that previously reported associations might largely result from residual confounding or reverse causation. Although testosterone assessment might improve risk prediction, implementation of testosterone replacement therapy requires further evidence of a direct effect on cardiometabolic outcomes from double-blinded randomized controlled trials and large-scale Mendelian randomization meta-analyses.

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P184

Adults with GH deficiency have subclinical longitudinal left ventricular dysfunction, without significant vascular function impairment, suggesting intrinsic myocardial disease

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Aims

GH deficiency (GHD) is associated with increased cardiovascular events. We aimed to evaluate cardiac, arterial, and endothelial function, by conventional and 2D speckle tracking echocardiography (STE), and biomarkers (proBNP and troponin I), in GHD patients by comparison with normal individuals with similar cardiovascular risk profile.

The study included 52 GHD patients (46.9 ± 15.6, 36 males), free of cardiovascular disease, severe hypertension or diabetes and 50 normals (N). Global LV systolic function was assessed from LV fractional shortening (FS), LV ejection fraction (LVEF), MAPSE, and cardiac index (CI). By STE, longitudinal LV function was assessed from: global longitudinal systolic strain (GLS), global longitudinal systolic, early and late diastolic strain rate (SRs, Sre, SRl); radial function from global radial strain (GRS); circumferential function from global circumferential strain (GCS); and LV torsion from basal (RotB) and apical rotation (RotA), LV torsion (LVtor), twist rate (TR) and untwist rate (UTR). Arterial function was assessed from intima-media thickening (IMT), local wave speed (LWS), Peterson elasticus modulus, and stiffness index (β); endothelial function from flow mediated dilation (FMD).

Results

In GHD patients, conventional systolic LV parameters were significantly decreased compared to N (FS: 28 ± 8.5 vs 38.6 ± 7.3; LVEF: 54 ± 8 vs 66 ± 8; MAPSE: 12 ± 2 vs 16 ± 2; CI: 2.1 ± 0.75 vs 2.7 ± 0.77, all $P<0.001$), but did not always exceed the normal range. 2D STE revealed significantly decreased GLS (-17.15 ± 2.7 vs -19.3 ± 3.3, $P<0.001$), and GCS (51.2 ± 17 vs -18.8 ± 3.24, $P=0.002$), with similar GRS. In addition, GHD had decreased values for systolic, early and late diastolic SR (SRs, SRe, and SRl), when compared to normal subjects (-0.9 ± 0.4 vs -1.1 ± 0.2, $P=0.016$; 1.15 ± 0.4 vs 1.31 ± 0.3, $P=0.027$; 0.71 ± 0.19 vs 0.96 ± 0.21, $P<0.001$). They also had lower RotB (-4.78 ± 2.6 vs -6.2 ± 2.1, $P=0.003$), LV torsion (1.8 ± 0.6 vs 2.3 ± 1.1, $P=0.011$), TR (92.8 ± 30 vs 121 ± 52, $P=0.002$), and UTR (99 ± 36 vs -132 ± 47, $P<0.001$), but similar apical rotation. Arterial and endothelial function parameters were similar between groups. While troponin I was normal in all patients, proBNP levels were significantly increased in the GHD group.

Conclusion

GHD patients have subclinical LV systolic and diastolic dysfunction, best revealed by STE, associated with increased proBNP levels and correlates to the level of GH deficiency ($r=0.614$, $P<0.001$). Our findings suggest that patients with GHD have intrinsic myocardial disease, probably due to insufficient contractile function of the myocardial fibres.

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P185

Mechanism of oxytocin-mediated cardiomyocyte protection

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Oxytocin (OT) treatment improves heart functional recovery in rat model of myocardial infarct. We investigated in the H9c2 cell line (cardiomyocytes model), mechanism of OT action in simulated ischemia - reperfusion (sI-R). H9c2 cells were suspended in warm ischemic buffer and placed inside an anoxic chamber for 2 h at 37 °C, then 'reperfused' under normal nutrients and oxygen conditions for 2 h. OT presence during ischemia increased cell viability by $9.7 \pm 2.5\%$ ($P<0.05$) and OT at reperfusion increased cell viability further by $15.8 \pm 3.5\%$ ($P<0.001$) as measured by MTT cell proliferation assay, OT treatment before ischemia had no effect. The significant cellular protection started at a concentration of 1 nM of OT, with an optimal protection at 62.5–125 nM. OT antagonist dose-dependently blocked the protective effect of OT. OT treatment of H9c2 cells transfected with OT receptor (OTR) siRNA increased cell apoptosis threefold in comparison to the control. OT increase in cell mortality in OTR siRNA cells could be mediated by vasopressin (AVP) receptors. Indeed, blockade

of AVP receptors by conivaptan increased cell viability in sI-R conditions. OT treatment reduced fluorescence of CM-H_2DCFDA products in sI-R-treated cells indicating decrease of reactive oxidative species (ROS). Interestingly, these experiments revealed that under normoxic conditions, OT treatment alone is sufficient to trigger a short-lived burst in intracellular ROS. The OT protection of sI-R was blocked by the PI3K–Akt inhibitor, Wortmannin. Using confocal microscopy, we noted that cells treated with OT displayed increased Akt (Thr308) phosphorylation and specifically, Akt was accumulated in ring-like structures associated with mitochondria and nuclei. Demonstration that KT5823, inhibitor of protein kinase G (PKG) and ODQ, inhibitor of soluble guanylyl cyclase, reduced OT-mediated protection in sI-R, indicated the role of cGMP-dependent protein kinase. Consequently, the confocal microscopy demonstrated the increase of eNOS phosphorylation and its nuclear accumulation in cells treated with OT. OT protection in sI-R was also inhibited by ANP receptor antagonist, A71915. These data suggest that OT provides protection to the injured heart by inhibition of ROS, cardiomyocyte apoptosis and stimulation of cell protective PI-3K–Akt–PKG signaling.

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P186

The effect of testosterone replacement on endothelial dysfunction, inflammation and insulin resistance in male hypogonadotrophic hypogonadism

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Objective
Metabolic disorders are common in patients with hypogonadism. Testosterone replacement therapy (TRT) significantly improves symptoms of testosterone deprivation such as mood, libido or musculoscelatal power. However, whether the metabolic and cardiovascular risk is improved is not clearly known. We aimed to search for the metabolic and cardiovascular effects of TRT.
Design
Prospective study, performed in the outpatient units of Gulhane Medical School.
Methods
Treatment naive young patients with congenital hypogonadotrophic hypogonadism (CHH; $n=80$, mean age 21.56 ± 2.1 years) were treated with either testosterone esters (250 mg/3 weeks i.m.; $n=59$) or testosterone transdermal gel (50 mg daily; $n=21$) in a mean follow-up period of 6.0 ± 2.4 months. The demographic parameters, fasting glucose, insulin, pentraxin 3 (PTX3) and asymmetric dymethyl arginine (ADMA) levels were measured both before and after treatment periods. The insulin sensitivity was estimated by HOMA-IR formula.
Results
The BMIs and waist circumferences were increased ($P<0.001$ and $P=0.001$ respectively) and the total and HDL cholesterol levels were decreased ($P=0.002$ and $P<0.001$ respectively) after the follow-up period. Both plasma ADMA and PTX-3 levels were increased ($P<0.001$ and $P=0.02$ respectively) while there were no significant alterations in the HOMAIR values. The alterations were similar in two different TRT regimens.
Conclusions
The study shows that, both daily transdermal and periodic injectable modalities worsen the surrogate markers of endothelial dysfunction and inflammation in young and treatment naïve subjects with CHH. Randomized prospective cohorts are warranted to see whether these short term unfavorable results will affect cardiometabolic outcomes of these patients.

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P187

TSH and arterial stiffness in healthy postmenopausal women

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Introduction
Thyroid dysfunction, whether overt or subclinical, has a significant effect on the cardiovascular system. On the other hand, fluctuation of thyroid hormones within the normal range has been associated with long-term health risks, and might have an impact on the vasculature. This study assessed the effect of thyroid hormones on surrogate markers of arterial structure and function in a sample of euthyroid postmenopausal women.
Methods
This cross-sectional study recruited 106 healthy postmenopausal women with thyroid stimulating hormone (TSH) levels within the laboratory reference range (0.4–4.5 μIU/ml). Blood samples were obtained for biochemical evaluation. Anthropometric measures and blood pressure were determined in each individual. Surrogate markers of vascular structure and function were assessed by intima-media thickness as well as pulse wave velocity (PWV), augmentation index and flow-mediated dilation, respectively. The associations between arterial markers and serum TSH, free thyroxin, free triiodothyronine, as well as serum thyroglobulin and thyroid peroxidise autoantibodies.
Results
A significant positive association was observed between mean measures of PWV and levels of TSH in quartiles (P value for linear trend 0.014). Significantly higher values of PWV were identified in subjects with serum TSH >2.5 μIU/ml when compared with subjects with TSH levels <2.5 μIU/ml (P value $=0.030$, univariate). In multivariate analysis, PWV was predicted by age, insulin resistance and TSH >2.5 μIU/ml (TSH, β-coefficient $=0.222$; P value $=0.014$). No associations were found between the remaining markers and levels of thyroid hormones, whereas thyroid antibodies were not associated with any of the arterial markers.
Conclusion
We demonstrated that serum TSH is an important predictor of arterial stiffness in euthyroid postmenopausal women. Women with TSH levels in the upper normal range have increased arterial stiffness compared to women with lower TSH. The upper limit of normal TSH in postmenopausal women may need reevaluation with respect to the effects on the vasculature.

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P188

A potential predict value of circulating osteoprotegerin in diabetic patients with asymptomatic coronary artery disease

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Background
Osteoprotegerin (OPG) is a bone-related glycopeptide produced by vascular smooth muscle cells due to arterial damage. OPG is considered as an independent predictor of cardiovascular disease in diabetic populations.
The aim of this study was to evaluate the interrelation between circulating OPG and coronary vasculature damage in type 2 diabetes mellitus patients.
Methods
One hundred and twenty six subjects with stable diabetes mellitus 2 type with previously angiographic documented asymptomatic CAD were enrolled to the study. Vessel-wall and plaque geometrical and compositional parameters were measured on contrast-enhanced CT angiography. The volume of intramural calcium of >320 HU in major coronary vessels was measured in 428 coronary segments with a highly standardized method. Coronary artery calcification was quantified by calculating the Agatston' score index and calcification mass measurement. OPG plasma levels were measured with ELISA.
Results
Circulating OPG level was increase in 105 patients (5201 pg/ml (95% CI = 3605–6280 pg/ml) and was normal in 21 subjects (880 pg/ml; 95% CI = 745–1140 pg/ml; $P<0.0001$). The relationship between coronary artery calcium by Agatston' score index and percent atheroma volume (PAV) was determined by linear regression. PAV and remodeling index were significantly higher in patients with elevation of OPG plasma level when compared with those who have normal OPG (adjusted odds ratio (OR) = 4.60 (95% CI = 2.23–14.50); $P=0.006$). There was significant correlation between Agatston' score index and PAV ($r^2=0.46$, $P=0.008$). Results did not change after adjustment for age, BMI, hypercholesterolemia, arterial hypertension, and exposure of antidiabetic drugs ($\beta=0.490$, 95% CI = 0.31–1.02, $P=0.282$). Comparable results were found for calcification mass. However, minimal luminal diameter and minimal luminal area were higher in patients with normal OPG plasma level ($P=0.002$).
In conclusion, we demonstrated that OPG plasma level can associate with vessel-wall thickening, percent atheroma volume, and Agatston' score index value in type 2 diabetes mellitus patients with previously angiographic documented CAD. Results from this study underline the importance of this biomarker use for screening procedure in diabetic populations aimed to specify of coronary vasculature damage severity and probably to recalculate cardiovascular risk.

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P189

Metabolic profile of transsexual persons on cross-sex hormonal therapy in a multi-center prospective intervention study

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Introduction
Gender differences in insulin resistance, body composition and lipid profile are well known and related to sex steroid hormones. In this study, we examine the metabolic profile of transsexual persons undergoing drastic sex steroid changes, during the first year of hormonal therapy.
Design
This research is part of a prospective intervention study conducted in several European gender teams (Ghent, Oslo, Amsterdam, and Florence).
Subjects
We present the data of Ghent gender team with 56 male-to-female (transwomen) and 24 female-to-male (transmen) transsexual persons, of whom 36 and 13 respectively have been in follow-up for 1 year of cross-sex hormonal therapy (CSH).
Methods
Standardized treatment regimens were used with oestradiolvalerate, 4 mg daily (or transdermal 100 μg/3 days for patients older than 45 years old) combined with cyproterone acetate 50 mg daily for transwomen and testosterone undecanoate i.m. 1000 mg/12 weeks for transmen. A glucose tolerance test was performed, HOMA-IR was calculated, waist-hip-ratio, lipids, total body fat and lean mass (dual X-ray absorptiometry), regional muscle mass and subcutaneous fat mass at the forearm and calf (peripheral quantitative CT-scan) and grip strength (hand dynamometer) were measured, before and after 1 year CSH.
Results
In transwomen, anti-androgens and oestrogens induced a higher total and subcutaneous fat mass and lower lean mass, muscle mass and strength and a lower waist-hip ratio (all $P\leq0.001$). Fasting insulin and HOMA-IR were higher after 1 year of CSH. HDL, LDL, and triglycerides decreased after 1 year (all $P\leq0.04$). Transmen gained lean body mass and muscle mass and strength and lost total body fat (all $P<0.001$) as well as subcutaneous fat after 1 year of testosterone ($P=0.019$). A decrease in HDL and increase in triglycerides was observed ($P\leq0.015$).
Conclusions
Oestrogen and anti-androgens in transwomen lead to more fat mass with a gynoid pattern of distribution. Testosterone treatment induces a less favourable lipid profile in transmen.

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P190

Changes in lipid profile and markers of metabolic syndrome after 3 years of testosterone-therapy in female-to-male transsexuals

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Introduction
Different studies have published that testosterone therapy in men and women results in decreased HDL cholesterol and increased LDL cholesterol, and therefore an increased cardiovascular risk.
Aims
To determine whether testosterone therapy has this effect on lipid parameters and markers of metabolic syndrome (MetSyn) in female-to-male transsexuals (FMT).
Material and methods
We studied 50 FMT, aged 27.8 ± 7.6 years, at baseline and after 3 years of treatment with testosterone undecanoate (1000 mg/12 weeks, i.m.). None had done gonadectomy. Weight (W), BMI, waist and hip circumference, and systolic and diastolic blood pressure were determined. We also measured plasma levels of glucose, total cholesterol (TC), LDL, HDL, tryglicerides, apolipoprotein A-I (ApoA-I), apolipoprotein B (ApoB), lipoprotein (a) (Lp(a)), homocysteine (Hcy), 25-hydroxy vitamin D3, iron, ferritin, transferrin, total testosterone, estradiol, prolactin, sex hormone-binding globulin, delta 4-androstenedione, dehydroepiandrosterone sulphate, FSH and LH.
Results
After 3-year testosterone-therapy, there was a significant increase in the levels of testosterone (52 ± 26–697 ± 277 ng/dl, $P=0.001$), TC (166 ± 29–180 ± 33 mg/dl, $P=0.031$), LDL (97 ± 27–113 ± 27 mg/dl, $P=0.027$), ApoB (79 ± 20–86 ± 20 mg/dl, $P=0.021$), iron (74 ± 38–96 ± 47 mg/dl, $P=0.031$), ferritin (44 ± 25–57 ± 32 mg/dl, $P=0.031$), and Hcy (10 ± 4–12 ± 3 mg/dl, $P=0.012$); and a significant decrease of HDL (53 ± 12–47 ± 11 mg/dl, $P=0.002$), ApoA-I (152 ± 25–135 ± 23 mg/dl, $P=0.001$), and Lp(a) (24 ± 20–18 ± 20 mg/dl, $P=0.041$). The other values unchanged significantly.
Conclusion
We conclude that long-term testosterone-therapy in FMT can promote an increased atherogenic by lowering HDL and ApoA-I, and by increasing TC, LDL, ApoB, Hcy, iron, and ferritin levels. But on the other hand, it produces a decrease in Lp(a) and without changing other markers of MetSyn.

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P191

Pentraxin-3 as a more sensitive marker of coronary artery disease severity than C-reactive protein

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Introduction
Pentraxin-3 (PTX3) is an acute-phase reactant that shares structural and functional homology with C-reactive protein (CRP). However, unlike CRP, which is synthesized mainly in the liver, PTX3 is produced at the site of inflammation. It has been suggested that PTX3 plays the same role in the periphery that CRP does in circulation. PTX3 may represent a rapid marker for local inflammation.
Methods
Fifty three patients with stable coronary artery disease (CAD) were enrolled. Coronary angiography was performed during index hospitalization because of the clinical presentation. Blood samples were collected on admission. Plasma concentration of PTX3 and high-sensitivity CRP (hsCRP) were determined.
Results
Median PTX3 concentration was 0.92 µmol/l (0.58–1.40). Median hsCRP concentration was 0.90 mg/l (0.75–1.10). There was a positive correlation between PTX3 and total cholesterol ($R=0.34$; $P=0.01$), PTX3 and LDL cholesterol ($R=0.35$; $P=0.01$), and PTX3 and hsCRP ($R=0.46$; $P=0.0005$). We found no correlation between hsCRP and all laboratory parameters. We found higher PTX3 concentrations in patients with Canadian Cardiovascular Society (CCS) functional class 2 (compared to CCS functional class 3), in patients with multi-vessel CAD (compared to single-vessel CAD), and in patients tasking nitrates. Lower PTX3 concentrations were reported in patients taking calcium channel blockers (amlodipine). hsCRP concentrations remained similar among these subgroups of patients. Conclusions: PTX3 was a marker of clinically more advanced CAD (CCS2 vs CCS3; nitrates vs no nitrates) and of a more severe atherosclerosis (single-vessel CAD vs multi-vessel CAD). PTX3 is also associated with other cardiovascular risk markers (total cholesterol, LDL cholesterol, and hsCRP). PTX3 may be a potential early marker of cardiovascular risk before the increase of systemic markers like hsCRP.

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P192

Effect of cross-sex hormone treatment on lipid profile in transsexual individuals: experience in a specialized unit in Catalonia

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Objectives
To assess baseline differences on lipid profile in individuals with gender identity disorder (male to female, M2F and female to male, F2M) in relation to prior cross-sex hormone treatment (CHT) or not, and changes in the lipid profile after 24 months of treatment and follow-up in our unit.
Methods
Transversal study by revision of medical records of 235 patients visited for first time in our service from 2006 to 2010. Clinical and analytical revision was made on the first visit, 6, 12, and 24 months.
Results
At baseline, F2M (92 patients, 39%) transsexuals were younger than M2F (143 patients, 61%) (27.9 ± 8.7 vs 32.0 ± 9.8 years; $P=0.03$), they had lower triglycerides levels (68.8 ± 29.7 vs 92.2 ± 71.4 mg/dl; $P=0.001$) and higher HDL levels (51.4 ± 12.3 vs 45.0 ± 11.7 mg/dl; $P<0.001$). No differences in baseline lipid profile was observed between individuals with previous hormonal treatment versus naive in none of the two groups, but in M2F group, individuals with previous treatment had a higher weight (71.8 ± 12.3 vs 67.0 ± 12.1; $P=0.038$).
In M2F transsexuals group, at 24 months follow-up, we observed a significant increase in weight (71.1 ± 13.0 vs 73.8 ± 5.2 kg; $P<0.001$) and BMI (23.6 ± 5.5 vs 24.5 ± 6.3 kg/m^2; $P=0.001$) without significant changes in the lipid profile.
On the other hand, at 24 months follow-up, in the F2M transsexuals group, we observed a significant increase in weight (67.2 ± 13.6 vs 69.8 ± 12.5 kg; $P=0.005$) and BMI (25.3 ± 5.0 vs 26.4 ± 4.1 kg/m^2; $P=0.002$) and a worsening of lipid profile with increased total cholesterol (168.5 ± 35.8 vs 178.2 ± 37.9 mg/dl, $P=0.003$), triglycerides (71.7 ± 31.4 vs 105.3 ± 70.2 mg/dl; $P<0.005$), LDL (106.0 ± 27.9 vs 115.2 ± 29.1 mg/dl; $P=0.018$), no-HDL cholesterol (118.7 ± 32.5 vs 136.3 ± 34.1; $P<0.000$) and decrease in HDL (52.8 ± 13.5 vs 45.4 ± 14.5 mg/dl; $P=0.001$).
Conclusions
CHT leads to changes in lipid profile at 24 months, highlighting the worsening in F2M transsexuals.

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HDL cholesterol subfractions and the effect of testosterone replacement in hypogonadism

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Metabolic disorders and cardiovascular events are increased in hypogonadism. Serum HDL composition is a better cardiovascular predictor than the HDL counts. However, there is no information about the HDL subfractions in patients with hypogonadism. We designed a prospective study to investigate the HDL subfractions in treatment naïve subjects with hypogonadism and the effects of two different testosterone replacement regimens on the HDL subfractions. Seventy young male patients with congenital hypogonadotropic hypogonadism (CHH) and 70 age and BMI-matched healthy males were enrolled in the present study. The patients were assigned to receive intramuscular injections of testosterone esters 250 mg every 3 weeks and transdermal testosterone applications 50 mg daily. Biochemical investigations including HDL subfractions and insulin resistance were done. Patients with CHH had higher levels of insulin, HOMA-IR, WC, triglyceride, and diastolic blood pressure. Although, the HDL cholesterol concentrations were similar in both groups, hypogonadal patients had lower HDL2 and higher HDL3 levels. The total testosterone levels were independent determinants of the HDL2 subfractions. During the follow-up, a significant increase in the BMI and WC values and a significant decrease in the levels of total cholesterol, HDL cholesterol and HDL3 were observed. No difference was present between the two treatment arms. These results show that low testosterone levels in hypogonadism may be associated with unfavorable HDL subfractions. Nevertheless, neither metabolic disorders nor the unfavorable HDL subfractions were able to improve with testosterone replacement therapy. The implications of these findings for the cardiovascular health should be sought with prospective follow up studies.

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P194

The activity of inflammation and the blood redox status in patients with coronary heart disease and type 2 diabetes mellitus

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To determine the relationship between indicators of inflammation and the blood redox status of type 2 diabetic (T2DM) patients with and without coronary heart disease (CHD).
Forty four patients with T2DM and CHD (group 1), 67 patients with T2DM without cardiovascular complications (group 2), 89 healthy subjects (group 3) were included in this study. Serum concentrations of IL6 and IL8 were determined by ELISA using commercial kits. Concentrations of thiobarbituric acid reactive substances (TBARS) both in plasma and in atherogenic lipoprotein, total glutathione (GSHt) and oxidized glutathione, as well as the activity of glutathione peroxidase and glutathione reductase in erythrocytes, and that of catalase in plasma were determined by spectrophotometric methods. The glutathione redox potential (E_h) was calculated by the Nernst equation.

The increased in IL6 concentration was found in group 1 (87%, $P=0.000$) and group 2 (20%, $P=0.007$) compared to group 3, the IL8 concentration was increased only in group 1 (56%). Decreased activity of catalase and glutathione peroxidase was revealed in groups 1 and 2 compared to group 3. While the decrease in GSHt and GSH concentrations was found only in group of patients with T2DM and CHD (58 and 71%, respectively). This group of patients was characterized by the increased values of E_h (36.3 mV, $P=0.000$) compared to the healthy subjects.

Consequently, patients with T2DM and CHD are different from diabetic patients without cardiovascular complications to have increased pro-inflammatory cytokine IL6, decreased antioxidant capacity of the erythrocytes glutathione system and increased E_h values. High cytokine concentrations and changes in glutathione level as well as E_h can be considered as prognostic markers for assessing the risk of CHD progression in diabetic patients.

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P195

Utility of N-terminal pro-brain natriuretic peptide in the evaluation of patients with high clinical probability of non-ST segment elevation acute coronary syndrome

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Introduction

The study aimed to analyze the efficiency of a concomitant N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac high-sensitivity troponin T (hs-cTnT) testing in diagnosing non-ST-segment-elevation myocardial infarction (NSTEMI) in patients initially negative for the standard fourth-generation assay of cardiac troponin T (cTnT), but with high clinical probability of non-ST-segment-elevation acute coronary syndrome (NSTE-ACS).

Methods

One hundred and eight patients, 57 (52.8%) men, mean age 61.3 ± 15.4, admitted for high-risk symptoms of NSTE-ACS in a time-interval <4 h after the symptoms onset, but with initial cTnT levels <0.01 ng/ml, were prospectively investigated. Plasma cTnT, NT-proBNP, and hs-cTnT were measured by electrochemiluminescence on admission, then 3 and 6 h afterwards. NSTEMI was considered at plasma hs-cTnT levels $\geq$ the 99th percentile cut-off (0.014 ng/ml), and a $\geq$20% dynamic variation within 6 h. Aortic dissection, pulmonary embolism, left ventricular hypertrophy, myocarditis, renal dysfunction and obesity were excluded by clinical, echocardiographic and biological data. Local Ethics Committee approved the study protocol and informed consent was signed by each patient. Statistics: MedCalc 12.2.1.0.

Results

Using a combination of hs-cTnT plasma levels $\geq$0.014 ng/ml and a $\geq$20% 6-h hs-cTnT dynamic plasmatic variation testing, NSTEMI was diagnosed within the cTnT 'blind interval' in 37 (34.26%) additional patients with high-risk symptoms of NSTE-ACS The aria under the receiver operating characteristic (AUC) for NT-proBNP in diagnosing NSTEMI was 0.68 (95% CI = 0.56–0.74), $P=0.0345$. The optimal cut-off NT-proBNP plasma level for the diagnosis of NSTEMI on the ROC curve was 205 pg/ml, with a sensitivity of 72.28 (95% CI: 51.72–84.41) and a specificity of 65.42 (95% CI: 48.78–77.68), a positive predictive value of 58.84 (95% CI: 42.44–68.82) and a negative predictive value of 74.82 (95% CI: 62.82–82.42).

Conclusion

A multimarker strategy using NT-proBNP concomitant with hs-cTnT testing on admission in the early diagnosis of NSTEMI in patients with high clinical probability of acute coronary syndrome showed only an additional value of NT-proBNP testing.

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P196

Perturbed sympatho-vagal balance in Turner syndrome: relation to phenotype and aortic dilation

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Objective

The risk of aortic dissection is 100-fold increased in Turner syndrome (TS). Increased blood pressure (BP) and heart rate is present as well as an increased risk of ischemic heart disease and diabetes. This study aimed to prospectively assess heart rate variability (HRV) in TS and its relation to aortic dimensions.

Methods

Adults with TS ($n=91$, aged 37.4 ± 10.4 years) recruited through the Danish National Society of Turner Syndrome Contact Group and an endocrine outpatient clinic were examined thrice (mean follow-up of 4.7 ± 0.5 years). Healthy controls ($n=64$, aged 39.4 ± 12.1 years) were examined once. Aortic dimensions were measured at nine positions using 3D, non-contrast and free-breathing cardiovascular-MRI. HRV measured by short-term spectral analysis (supine-standing), transthoracic echocardiography and 24-h ambulatory BP were done.

Results

The changes in High frequency (HF) power (vagal activity) and low-frequency:high-frequency ratio (sympatho-vagal balance) was diminished in TS compared with controls when assessed by a two-way analysis of variance for the interaction term 'Position (supine-standing) $\times$ status (TS or control)' ($P<0.001$). HF was lower while supine ($P=0.001$) and higher while standing ($P=0.09$) in TS compared to controls. Aortic diameter was inversely correlated with LF (r-average $=-0.337$ and -0.334, supine and standing; $P<0.05$) and HF (r-average $=-0.405$ and -0.293, supine and standing; $P<0.05$) in controls. Same degree of correlation was present in TS: LF (r-average $=-0.312$ and -0.341; $P<0.05$) and HF (r-average $=-0.330$ and -0.307; $P<0.05$). Changes in aortic diameter did not correlate with any measures of HRV. Prospectively there were no changes in HRV.

Conclusions

A perturbed sympatho-vagal balance is present in TS explained by a decreased vagal activity in the supine position and increased vagal activity in the standing position. LF and HF correlate with aortic diameter in both groups, however no relation was found with changes in aortic diameter.

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P197

Impact of subclinical thyroid dysfunction on mortality among patients presenting with cardiovascular events

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Introduction

The possible clinical impact of subclinical thyroid dysfunction on patients with cardiovascular events is important if one considers the high incidence of a mildly altered thyroid hormone pattern in this population. This study aims to determine the prevalence of subclinical thyroid dysfunction among patients presenting with cardiovascular events in Philippine General Hospital, and its impact on mortality.

Methodology

A cross sectional, prospective cohort study, involving 163 adult patients with cardiovascular events. Pregnant, and all patients who have clinically apparent thyroid disease or taking thyroid medications, or any other medications that may affect thyroid function testing were excluded. Patients were grouped as having overt thyrotoxicosis, subclinical hyperthyroidism, euthyroidism, overt hypothyroidism, subclinical hypothyroidism, or nonthyroidal illness. Demographic and clinical characteristics were expressed as means. Cardiac and overall deaths were considered. Calculations were done using the SPSS program, version 20.0.

Results

Patients with subclinical hyperthyroidism were older (66.9 vs 56.9 years, P value $=0.0020$), and more were diabetic (55.6 vs 23.6, P value $=0.039$), while those with subclinical hypothyroidism and nonthyroidal illness have a higher need for mechanical ventilation (33.3 vs 9%, P value $=0.026$; 25.7 vs 9%, P value $=0.015$ respectively), compared to euthyroid. Prevalence of subclinical thyroid dysfunction was 32.5%. Of these, 5.5% had subclinical hypothyroidism, 5.5% had subclinical hyperthyroidism, and 21.5% had nonthyroidal illness. Higher incidence of acute coronary event and heart failure among patients with subclinical thyroid dysfunction (P value $=0.031$) was noted. The overall death rate was also higher (17.3 vs 9.2%).

Conclusion

In patients with cardiovascular events, the prevalence of subclinical thyroid dysfunction was 32.5%. Higher incidence of acute coronary event and heart failure was observed in the population. Subclinical hyperthyroidism was

significantly associated with older age and diabetes, while subclinical hypothyroidism and nonthyroidal illness with need for mechanical ventilation.

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Abstract withdrawn.

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P199

Lipid levels in patients with rheumatoid arthritis and the effect of rituximab

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Rheumatoid arthritis (RA) is known to be associated with cardiovascular comorbidity. In particular, patients with RA are known to be at increased for the development of atherosclerosis. Treatment with biological agents in RA may affect lipid levels.

Aim

The aim was to study the effect of rituximab treatment on lipid levels in RA patients.

Methods

In a cohort of 20 patients with RA lipid levels were studied before, 6 and 12 months after treatment with the biological agent rituximab (2×1000 mg i.v. infusions 2 weeks apart) at baseline, 6 and 12 months later. All patients fulfilled the 2010 ACR/EULAR criteria for RA. Total cholesterol, HDL, LDL cholesterol, and triglyceride levels were measured at baseline, 6 and 12 months later.

Results

At baseline total cholesterol was 207.41 ± 8.33 mg/dl (mean $\pm$ S.E.M.), 6 and 12 months later increasing to 218.27 ± 7.02 and 226.12 ± 8.71 mg/dl respectively ($P<0.001$, Student's t-test). At baseline HDL cholesterol was 59.17 ± 3.31 mg/dl, 6 and 12 months later increasing to 66.05 ± 3.49 and 58.81 ± 3.04 mg/dl, respectively ($P<0.001$). At baseline LDL cholesterol was 122.89 ± 8.96 mg/dl, 6 and 12 months later increasing to 124.58 ± 5.61 and 141.56 ± 7.95 mg/dl respectively ($P<0.001$). At baseline triglyceride levels were 138.50 ± 13.91 mg/dl, 6 and 12 months later decreasing to 122.55 ± 10.52 and 118.65 ± 9.91 mg/dl respectively ($P<0.001$).

Conclusion

Treatment with the biological agent rituximab in RA resulted in an increase in total cholesterol, paralleled by an increase in HDL cholesterol, LDL cholesterol, while triglyceride levels decreased. The adverse effect on total cholesterol levels may be counteracted by the parallel increase in HDL thus conferring a beneficial effect on the patients as far as cardiovascular risk is concerned. These findings have therapeutic implications as the effect of rituximab on lipid levels may render systematic treatment with statins necessary in rheumatoid arthritis.

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P200

Linear growth and IGF1 concentrations in children cyanotic and acyanotic congenital heart disease before vs after intervention

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Objectives

To measure linear growth of patients and the magnitude of catch-up growth and changes and IGF1 concentration.

Design

This prospective study recorded the anthropometric data and measured the IGF1 of 32 infants and children with congenital acyanotic heart disease (CAHD) with left to right shunt (14 VSD, 10 ASD, and 8 PDA) without heart failure, and 15 with cyanotic heart disease (CCHD) before (B) and a year after (A) surgical or catheter intervention.

Results

At presentation patients with CHD height SDS (HtSDS) and GVSDS were significantly decreased vs normal controls. The BMI of CCHD were significantly lower than normal controls. Before intervention, CCHD had significantly decreased length SDS (LSDS) and growth velocity SDS (GVSDS) vs CAHD. One year after intervention the HtSDS, GVSDS increased significantly in patients with CCHD and CAHD. After intervention CCHD had higher GVSDS compared to children with CAHD. BMI increased significantly in patients with CCHD after intervention. IGF1 levels increased significantly in both groups of patients after intervention. The HtSDS after treatment was correlated with the IGF1 concentration ($r=0.70$, $P<0.001$). In CAHD the shunt size was correlated negatively with BMI before intervention ($r=0.39$, $P<0.01$). GVSDS after intervention surgery was correlated with age at operation ($r=-0.62$, $P<0.001$) and BMI after intervention ($r=0.57$, $P<0.001$).

Table 1

Patients (n)	CCHD-B=15	CCHD-A=15	CAHD-B=32	CAHD-A=32	Controls-B=50	Controls-A=50
Age mon	11.9	24.6	30	43.2	18.5	32.4
LSDS	−2.6	−1	−1.1	−0.55	−0.2	−0.1
GVSDS	−1.3	3.5	−0.7	2.5	0.31	0.25
BMI	14.5	16.4	15	15.7	16.3	16.7
IGF1 (ng/ml)	41.5	72.3	53	79	ND	ND

Conclusions

These data denoted that early surgical interference and good weight gain have beneficial effect on postoperative growth spurt.

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P201

Changes of expression of regulators of calcification in different stages of atherosclerosis in vasculature and bone

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Calcification is physiologically present in bone but also pathophysiologically in the vasculature, favouring cardiovascular diseases. Our aim was to investigate changes in the expression of calcification regulators (CR) during vascular calcification in bone and vasculature. Gene expression levels of OPG, RANKL, OPN, MGP, BSP-II and RUNX2 were determined in bone, aorta and arteria ilica externa tissue samples of 22 transplant donors. Gene expression levels during atherosclerotic changes in the vessels were investigated using three histological stages of atherosclerosis (0) no changes, (1) intima thickening or (2) intima calcification. Patients' other tissue samples were subgrouped accordingly. In addition, serum measurements of PTH, Fetuin A, 25(OH)Vitamin-D3 and $1,25(OH)_2$-Vitamin-D3 were performed.

Results

The comparison of gene expression of CR in vascular tissue revealed that the expression of CR was already changed in thickened vessels and kept stable during calcification. Therefore, we compared unaffected with affected vessels. We demonstrated that the expression of BSP-II and OPN were significantly ($P=0.034$) and RANKL expression was by trend decreased ($P=0.085$) in affected vessels compared to unaffected ones. In comparing bone and vascular tissue, patients with no atherosclerosis (stage 0) showed no differences in CR expression in bone and vascular tissue. In stage 1 patients, expression of MGP ($P=0.002$) and OPG ($P=0.001$) was significantly higher in bone than in both vessel types, whereas in stage 2 patients, OPG expression increased in both vessel types. Serum levels of Fetuin A were significantly lower in stage 2 patients compared to the other stages ($P=0.007$).

Conclusion
Our study indicates that changes in the expression of CR in the vessel wall as well as in bone tissue occurs already in the stage of thickening of the vessel wall, even prior to deposition of calcium/phosphate precipitation.

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P202

Dyslipidemia associated with m-TOR inhibitors treatment

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Introduction
Therapeutic approach of patients (pts) with metastatic renal cell carcinoma (mRCC) may include the use of biological agents such as m-TOR inhibitors: temsirolimus (TM) and everolimus (EV). Its use is associated with metabolic dysfunction, especially with everolimus: hyperglycemia (37% TM treated pts vs 72% EV treated pts), hypercholesterolemia (25% TM treated pts vs 81% EV treated pts) and hypertriglyceridemia (30% TM treated pts vs 73% EV treated pts). Discontinuation of therapy is suggested, if grade 4 toxicity is observed: blood glucose $>$ 500 mg/dl, total cholesterol (TC) $>$ 500 mg/dl and triglycerides (TG) $>$ 10 $\times$ UNL.
Objective and methods
Retrospective evaluation of lipid profile (LP) in pts with mRCC treated at our institution with m-TOR inhibitors (TM and EV), between June 2010 and August 2012. We evaluated TC and TG levels. Pts with hypothyroidism (previous treatment with sunitinib) and pts without baseline LP were excluded.
Results
We evaluated five pts, 4♂, 1♀, aged between 55 and 70 years. Three patients were treated with TM and two with EV. All patients had dyslipidemia at baseline. Average TC and TG values were assessed before treatment: CT 224 $\pm$ 54 mg/dl (191 mg/dl – TM group vs 275 mg/dl – EV group); TG 247 $\pm$ 115 mg/dl (202 mg/dl-TM group vs 313 mg/dl-EV group). After therapy (med. 2.4 months): TC 278 $\pm$ 137 mg/dl (+24%), TG 557 $\pm$ 383 mg/dl (+126%). TC's increase in the group of pts treated with TM and EV was +25% (with TM) vs +23% (with EV), and TG's increase was +101% (with TM) vs +150% (with EV).
Conclusions
Therapy with m-TOR inhibitors in this small number of pts was associated with an increase in the lipid parameters assessed, especially TG. Metabolic toxicity in patients on TM and EV should be promptly addressed and requires an interaction between health professionals in order to develop evaluation and follow-up protocols.

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P203

Rising prevalence of fatty liver in India and its correlates

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Apollo Hospitals, Chennai, India.

Among individuals who came for master health check up to Apollo hospitals, Chennai between June 2011 to November 2011, 750 patients were recruited based on the inclusion and exclusion criteria. Those who showed fatty liver on ultrasonography were compared with those who had normal liver parenchyma. People with acute medical illness, known hepatic disease, known renal disease, people who consume excessive alcohol ($>$ 20 g/day), patients on medications that cause hepatotoxicity (such as estrogens, corticosteroids, amiodarone, valproate; at present or within the last 2 years) were excluded.
Our study showed 44% prevalence of fatty liver, which is much higher than those reported in last decade and certainly indicates an upward trend in increasing prevalence of FLD (earlier studies have shown prevalence anging from 10 to 22%). In this study FLD was present mainly in the middle-aged group with peak occurrence around 40–50 years with mean age at presentation being 47 $\pm$ 11 years. Of them FLD was more common in men and this might be due to higher waist–hip ratio in men as compared to women, which is an indicator of central obesity and insulin resistance. We also found that fatty liver was undoubtedly associated with high BMI, increased waist circumference and WHR. Eight percent of the patients with FLD were either overweight or obese (49 and 31% respectively). As increased ALT has been reported to be positively associated with FLD, we also found that 30% of patients with FLD had elevated AST ($>$ 35 U/l) and ALT ($>$ 45 U/l) with the mean ALT greater than AST (44 $\pm$ 32 vs 34 $\pm$ 20). Seventy-two percent had ALT: AST ratio greater than the reverse ratio in FLD group (22%). Also, 76% of FLD group were either diabetic (36%) or pre-diabetic (40%) with only 24% being normal. The mean fasting and 2 h postprandial blood sugar levels in FLD patients were markedly higher than the control group (122 $\pm$ 45 and 167 $\pm$ 83 respectively). In this study, though the occurrence of hypertension was not significant, there was significant increase in the mean systolic BP in FLD patients (130.37 $\pm$ 16.59). Seventy-three percent of our patients with FLD had metabolic syndrome. This is higher than that reported in a study conducted in South India. When compared to the last decade, the overall prevalence of FLD is progressively increasing in India in parallel with the increasing prevalence of T2DM, obesity and metabolic syndrome.

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P204

CNR1 polymorphisms and metabolic disorders in woman with PCOS-preliminary report

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The endocannabinoid system has been suspected to contribute to the association of visceral fat accumulation with metabolic diseases. Insulin resistance play important role in etiology in PCOS. The aim of our study was developed the role of CNR1 polymorphisms (rs806368, rs12720071, rs1049353, 806381, rs10485170, rs6454674, rs2023239) in metabolic disorders in woman with PCOS. 88 woman (20–35 years old) with PCOS (recognized using Rotterdam criteria) and 77 woman (20–35 years old) as homogenous controls group were study. Lipids profile and carbohydrate metabolism in serum using standard methods were study. Genomic DNA were isolated using standard method. To amplify the genetic material of the PCR technique was used. To polymorphisms identification minisequencing was used. Reaction products were separated on Genetic Analyser ABI 3100. For statistic analysis ANOVA test was used.
In polymorphism rs12720071 eight woman with PCOS present genotype G/G in contrast to control we can't find such a genotype. These genotype in polymorphism rs1049353 in PCOS group present a higher WHR vs other genotype in contrast to control group. Also other polymorphism rs 10485170 in genotype A/G show significantly lower LDL cholesterol levels in controls but not in woman with PCOS. In polymorphism rs2023239 genotype C/C was connected with elevated levels of free androgens index in PCOS patients.
Our preliminary results can suggest the potential role of CNR1 polymorphisms PCOS etiology what need further study.

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P205

Oleic vs linoleic effects on hepatic sex hormone-binding globulin production

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Low plasma SHBG is an independent risk factor of cardiovascular disease. Our recent work has shown that SHBG gene expression can be regulated indirectly through HNF4α gene expression. In this regard, we have identified that an

increase in hepatic palmitate induced by high carbohydrate diets and pro-inflammatory cytokines (TNFα and IL1β) was able to reduce hepatic HNF4α protein levels which, in turn, decreased SHBG production. The Mediterranean diet, in which olive oil is the primary source of fat, is associated with a lower risk of cardiovascular disease. Since olive oil is the primary source of fat intake, the Mediterranean diet is high in monounsaturated fatty acids (MUFA), specifically oleic acid. The purpose of this study was to investigate the effects of oleic and linoleic acids in the regulation of SHBG production in HepG2 cells and to examine the underlying molecular mechanisms. We provide evidence that oleic acid treatment increased SHBG (mRNA and protein) when compared with linoleic acid treatment over the course of three days in HepG2 cell cultures. Oleic acid and linoleic acid treatment did not change HNF4α (mRNA and protein levels). However, oleic acid treatment reduced PPARγ (mRNA and protein levels), a well-recognized SHBG inhibitor, when compared with linoleic acid treatment. Finally, oleic acid treatment produced a reduction in PPARγ binding and an increase in HNF4α binding to the SHBG promoter when compared with linoleic acid treatment in ChIP assays. In conclusion, our results provide evidence that treatment with oleic acid increases SHBG production, mainly by down-regulating PPARγ. Our findings suggest that the effect of oleic acid in increasing SHBG could be a new mechanism involved in the benefitial effect of Mediterranean diet on cardiovascular disease.

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P206

Post prandial responses in insulin, free fatty acid and endothelial function following to Malaysian vs Mediterranean meals among healthy subjects

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Introduction
Refined carbohydrates, which constitute the staple diet in most cultures may exaggerate postprandial responses in term of insulin, free fatty acid and vascular function. These changes may contribute the onset of metabolic syndrome and diabetes among those at high risk of developing these diseases.
Objective
To determine the postprandial insulin, free fatty acids levels and endothelial function responses in healthy subjects to two different breakfast meals, Mediterranean vs Malaysian diet with different carbohydrate contents, glycaemic index and glycaemic load.
Methods
Twenty subjects were made to take two different meals, Mediterranean meals; low Glycaemic Index (GI) and low Glycaemic Load (GL) and Malaysian meals (high GI and high GL) a week apart. Blood parameters including fasting serum lipid, serum insulin, serum non esterified free fatty acid were taken at 0, 30, 60, 90, and 120 min after each meals. Blood glucose was also taken at baseline and 120 min postprandially.
Results
Twenty subjects were randomized to either Mediterranean meal or Malaysian meal. Baseline clinical and demographic data parameters were comparable for both meals. With both meals there was an increased in serum triglyceride with a corresponding drop in HDL and LDL levels throughout the 2-h postprandial period. There is a significant increase in the serum insulin level with Malaysian meal ($P=0.002$) compared to Mediterranean meal ($P=0.012$). However, the non esterified fatty acid levels were significantly lower in Malaysian meal ($P=0.001$) compared to Mediterranean meal.
Conclusion
Malaysian meal which is characterised by high GI and GL stimulated significant increase in insulin responses in the setting of significant drop in free fatty acid compared to Mediteranean meal.
Key Words
insulin; serum nonesterified fatty acid (NEFA).

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P207

Subclinical atherosclerosis and cardiovascular risk in healthy, young menopausal women

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Introduction
Delayed diagnosis of a cardiovascular event is related with higher case-fatality rate in women as compared to men. In fact, accumulating evidence supports an association between the menopausal status and the development of cardiovascular disease (CVD). We aimed to assess the extent of subclinical vascular disorders in young, healthy postmenopausal women, with respect to the calculated cardiovascular risk.
Methods
This cross-sectional study recruited 120 healthy, young postmenopausal women without clinically overt CVD or diabetes, classified as not high-risk by the Heartscore. In addition to risk factors used for Heartscore calculations, we assessed menopausal age and associated metabolic risk factors (e.g. triglycerides, waist circumference, fasting blood glucose, and HOMA-IR. Carotid–femoral pulse wave velocity, carotid and femoral intima–media thickness in the abnormal range as well as atheromatous plaques both in carotid and femoral arteries were used to define the presence of subclinical atherosclerosis.
Results
Subclinical atherosclerosis was identified in up to 55% of women. In addition, presence of at least one plaque in carotid arteries was identified in up to 22.5% of women. Subjects with subclinical atherosclerosis had higher age and menopausal age, blood pressure and HOMA-IR. By multivariate analysis menopausal age (P value $=0.007$) and systolic blood pressure (P value $=0.021$) independently determined subclinical atherosclerosis while 79% of intermediate-risk women (Heartscore 2–4.9%) being in menopause for at least 4 years would be reclassified to a higher risk for the presence of atherosclerosis.
Conclusion
Subclinical atherosclerosis was highly prevalent in postmenopausal women with low to medium Heartscore. This discrepancy between the prevalence of subclinical atherosclerosis and calculated risk may be related to the risk factors determining atherosclerosis in this population, which are not included in HS calculations, like the menopausal status.

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P208

Comparative effects of atorvastatin and rosuvastatin on vitamin D levels, glucose metabolism and systematic inflammation in non-diabetic patients with dyslipidaemia: a prospective randomised open-label study

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Introduction
Low levels of 25-hydroxy-vitamin D [25(OH)D] have been recognized as a new cardiovascular disease risk factor. Conflicting data exist regarding the effect of statins on 25(OH)D levels and glucose metabolism.
Methods/design
This was an open-label randomized prospective comparative study evaluating the effects of atorvastatin and rosuvastatin at equivalent doses on 25(OH)D levels, glucose homeostasis and systemic inflammation in non-diabetic patients with dyslipidaemia. Fifty-two patients were randomly assigned to atorvastatin 20 mg/day ($n=28$, aged 56.1 ± 2.2 years, 22 females) or rosuvastatin 10 mg/day ($n=24$, aged 57.4 ± 1.9 years, 20 females). Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting plasma glucose, insulin, homeostasis model assessment-insulin resistance (HOMA-IR), glycosylated hemoglobin A_{1c} (HbA_{1c}) and high-sensitivity C-reactive protein (hsCRP) levels were measured at baseline and after 12 weeks. There were no differences in baseline characteristics between the two groups.
Results
Both statins significantly reduced TC, TG and LDL-C levels. The reduction in LDL-C was greater with rosuvastatin (49.4 vs 41.7%, $P=0.015$).
The increase in 25(OH)D levels with both statins was not statistically significant (from 21.7 ± 1.9 to 23.5 ± 2.3 ng/ml with atorvastatin ($P=0.205$) and from 25.3 ± 1.8 to 27.0 ± 2.4 ng/ml with rosuvastatin ($P=0.306$)).
Rosuvastatin was associated with a significant reduction in insulin levels (from 6.7 ± 0.8 to 5.2 ± 0.7 μIU/ml, (-8.5%), $P=0.048$)), although not in HOMA-IR. The respective changes with atorvastatin were not significant. The effect of both statins on fasting glucose and HbA_{1c} levels was neutral.
Regarding systematic inflammation, only atorvastatin significantly reduced hsCRP levels (from 4.1 ± 1.4 to 3.0 ± 0.7 mg/l (-13.5%), $P=0.025$).
Conclusions
Statins did not affect 25(OH)D levels in the present study. Rosuvastatin was associated with a reduction in insulin levels without affecting insulin resistance, while the effect of atorvastatin on glucose homeostasis was neutral. However, atorvastatin, led to a significant reduction in systematic inflammation compared with rosuvastatin.

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P209

Clinical experience with exenatide and liraglutide in the internal medicine service two of hospitals in Valencia

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Background and objective
The upcoming new marketing presentations GLP1 analogues with a dosage more comfortable for patients, we wanted to revise the two we currently have on the market. The target of this study is to determinate glicated hemoglobin (HbA1c) at 6 months of treatment, changes in weight, body mass index (BMI) and tolerance.
Design and methods
Observational study that included 60 patients with diabetes mellitus type 2 followed in two Internal Medicine Service. The inclusion of patients was consecutive until achieve 30 patients with each of the treatments. Dose reached: Exenatide 10 μg twice daily and Liraglutide 1.2 mg daily. Determinations baseline and 6 months of treatment.
Results
– Baseline data: Exenatide/Liraglutide (weight 102.61/105.73, BMI 38.19/41.35, fasting glucose 189.56/183.35, HbA1c 8.9 ± 0.6/8, 8 ± 0, 8).
– After 6 months with exenatide, the weight loss was 2.99 ± 5.12 kg ($P<0.01$), BMI reduction 1.36 ± 1.18 kg/m^2 ($P<0.0001$), of HbA1c $0.84\%\pm2.06$ ($P<0.05$) and of fasting glucose 23.63 ± 59.12 mg/dl ($P=0.09$).
– After 6 months with Liraglutide, the weight loss was 3.18 ± 3 kg ($P<0.05$), BMI reduction 1.18 ± 1.67 kg/m^2 ($P<0.05$), of HbA1c $0.9\%\pm1.9$ ($P<0.05$) and of fasting glucose 27.31 ± 73.18 mg/dl ($P<0.05$).
– Further reduction BMI (0.18 ± 0.77 kg/m^2) in the group with Exenatide ($P>0.05$).
– Greater weight loss (0.19 ± 2.18 kg), greater reduction fasting glucose (21.18 ± 3.68 mg/dl) and a HbA1c greater reduction ($0.06\%\pm0.610$) in the group with Liraglutide ($P>0.05$).
– 10 patients (33.3%) had gastrointestinal intolerance in the group treated with Exenatide and 4 (13.33%) in the group treated with Liraglutide (3 gastrointestinal intolerance and 1 dizziness).
– 5 dropouts for gastrointestinal intolerance and insuficient metabolic control in the group with Exenatide and 3 in the Liraglutide group.
Conclusions
Both drugs have very interesting clinical results in both efficiency (comparable to DPP4) and scarce secondary events. Notably Liraglutide currently offers an additional advantage for their a daily dosing.

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P210

Effect of one session resistance exercise on C-reactive protein and cardio metabolic risk factors in trained and untrained healthy students

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Introduction
We designed this study, to investigate the effect of a single resistance exercise session on serum C-reactive protein (CRP) and cardiometabolic riskfactors in trained and untrained young healthy male students and to evaluate whether regular training may affect the response of these markers to exercise.
Method
According to training status 34 healthy male students divided to 19 trained and 15 untrained groups. Each group was then randomly divided to intervention and control groups. The final groups included experimental trained (ET), control trained (CT), experimental untrained (EU) and control untrained (CU). The experimental groups underwent exercise training that consisted of 120 min intensive resistance program at 70–80% of 1 RM. The blood samples were collected just before the start of training program and 4 h post exercise to evaluate CRP and other metabolic markers.
Results
ANOVA analysis showed the serum level of blood sugar, insulin, components of lipid profile and CRP were not significantly different between four groups at baseline. More over 4 h post exercise CRP concentration and other cardio vascular riskfactors did not differ between 4 groups. Additionally the paired *t*-test showed the serum level of CRP and metabolic risk factors was not changed in response to acute resistance exercise in any group.
Conclusion
Our results indicate that one session of acute resistance exercise had no effect on serum concentration of CRP and cardio metabolic risk factors in healthy male students, no matter they were trained or untrained.
Key Words
resistance exercise; C– reactive protein (CRP); cardio metabolic markers; trained; untrained.

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P211

Combination therapy in diabetic patients with insulin glargine and exenatide in the internal medicine service two of hospitals in Valencia

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Background and objective
Combination therapy in diabetic patients is seeking better clinical outcomes by acting on different targets in the pathophysiology of the disease.

Insulin glargine provides excellent glycemic control, especially in the fasting glucose control, in spite of a increasing in weight, compared with exenatide which is more effective in controlling postprandial glycemic and in reducing insulin resistance, plus a reducing effect on the weight.
Design and methods
– Men/Women: 12/18, age 67.36±4.4 years, duration of diabetes 19.6±4.7 years.
– Baseline data: weight 106.51±7.24 kg, BMI 41.25±3.1258 kg/m2, glicated hemoglobin (HbA1c) 8.8±0.61, 64.97± insulin dose 18.64 UI.
– Changes in insulin requirements UI/kg: baseline 0.61±0.18, at 3–6 months 0.39±0.06 and 0.23±0.36 at 9-12 months. Significant reduction in insulin dose: 35.65% at 3–6 months and 48.65% at 9–12 months. Insulin was removed in five patients.
– Changes in weight: all patients lost weight: 4.6±2.73 kg at 3–6 months and 8.4±6.23 kg at 9–12 months.
– HbA1c evolution: reduced by 83.2% at 3–6 months and 85.3% at 9–12 months. The mean reduction in HbA1c was 0.9±1.03 at 3–6 months and 1.3±1.22% at 9–12 months.
– Tolerance: withdrawal for Exenatide in four patients (13.3%): 2 gastro-intestinals intolerance and another 2 by insufficient metabolic control.
Conclusions
The combined use of insulin glargine and exenatide improves glycemic control and in many cases allows the reduction of insulin dose, obtaining further reduction in weight, which makes this partnership a first-choice treatment in of patients with type 2 diabetes and BMI over 30 kg/m2.

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P212

Metabolic disorders in a group of Algerian hypersomatotropic subjects

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Introduction
Glucose metabolism disorders are well known in subjects with GH excess, but little is known about other metabolic abnormalities. Our aim is to analyze metabolic complications in subjects with acromegaly and pituitary gigantism.
Subjects and methods
Records of 111 subjects were retrospectively examined, mean GH = 62 ng/ml ($n < 5$), mean age = 39.11 (14–60). All had routine analysis for fasting blood glucose, cholesterol, triglycerides, calcium and phosphorus. They all had oral glucose tolerance test (75 g glucose) with glucose measurement during two hours. Hormonal assessment was based on GH and IGF1 (when possible), prolactin, cortisol, ACTH, TSH, FT4, FSH, LH, and testosterone or estradiol (according to their sex). Radiological exploration was based on CT scan, MRI or both.
Results
Among this group 44.8% had gonadotrop deficiency, 22% thyreotrop and 21% corticotrop deficits, because of their pituitary tumours (mean volume 11.4 cm). For metabolic disorders: 58.5% had glucose metabolism disorders, 54% hypercholesterolemia and/or hypertriglyceridemia, 31% hyper phosphataemia, and 10% hyperparathyroidism.
Conclusion
Our subjects, living in a developing country, with GH excess, are at very high risk of cardiovascular diseases because of their metabolic disorders, especially glucose and lipids abnormalities which are higher than recent Poland and Belgium studies in Europe.

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Clinical case reports – Pituitary/Adrenal

P213

Case report: chronic adrenergic stimulation induces brown adipose tissue differentiation in visceral adipose tissue

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Introduction
The presence of brown adipose tissue (BAT) in humans has recently been demonstrated by histology and positron emission tomography (PET). In previous reports, BAT in adults is primarily confined to the upper chest and neck. Here, we report a case of massive BAT infiltration of the visceral adipose tissue in a patient with catecholamine secreting paraganglioma.
Methods
The patient was evaluated with [^{18}F]-FDG PET/CT at three occasions: pre-therapy, during α-blockade and postoperatively. During surgery, biopsies of visceral and subcutaneous adipose tissue were obtained. Histological specimens were evaluated for BAT and uncoupling protein 1 (UCP-1) gene expression was verified by QPCR, WB and immunohistochemistry. Resting energy expenditure was measured with indirect calorimetry.
Results
At diagnosis, BAT glucose uptake assessed by FDG PET was massively increased (see figure). FDG uptake was confined to known locations for BAT with additional uptake in the visceral adipose tissue. No excess activity was observed in subcutaneous adipose tissue. Histological examination revealed areas of BAT in the visceral adipose tissue with ∼200-fold greater UCP-1 gene expression compared to subcutaneous adipose tissue. Owing to increased thermogenesis, resting energy expenditure was 15 188 kJ/day at diagnosis with a decrease to 8368 kJ/day after surgery. During α-adrenergic receptor-blockade, FDG uptake was decreased (see figure). After surgical removal of the tumor, BAT FDG uptake returned to normal (see figure). Normetanephrine level was greatly increased at diagnosis, with normalization after surgery (diagnosis: 3318 ng/l; post-surgery: 132 ng/l ($<$200 ng/l)).
Conclusion
In this case report, chronic adrenergic stimulation increased BAT differentiation and activity in visceral adipose tissue. Stimulation of adrenergic receptor signaling in BAT progenitor cells may have promising perspectives for future treatment of obesity and type 2 diabetes.

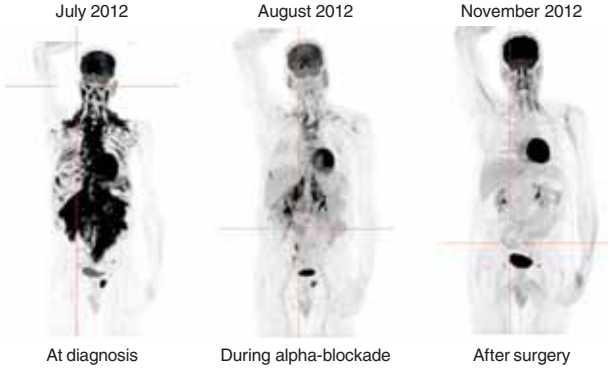

Figure 1

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P214

An unusual case of Cowden-like syndrome, neck paraganglioma and pituitary adenoma

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Introduction
The description of a rare case of papillary thyroid cancer, neck paraganglioma, pituitary adenoma and Cowden-like syndrome.
Case report
A 43-year-old woman presented with enlargement of her right thyroid lobe and a palpable ipsilateral neck mass. The pathology examination after operation of the two lesions revealed the synchronous presentation of a papillary thyroid carcinoma and a neck paraganglioma. Patient's medical history included a microprolactinoma, diagnosed one decade before and a constellation of characteristics that are components of Cowden syndrome, specifically an excised mammary gland fibroadenoma on grounds of fibrocystic disease and a large uterine leiomyofibroma under observation. Physical examination revealed macrocephaly and multiple skin papules.
Germline mutation analysis of *PTEN, SDHB*, *SDHC* and *SDHD* was performed with revelation of 3 polymorphic sites in introns 1, 4, 8 of PTEN gene and 1 polymorphic site in exon 1 of SDHB gene, but absence of known pathogenic mutations.

Conclusions
The co-existence of Cowden-like syndrome, neck paraganglioma and pituitary adenoma is described for the first time, which could represent a novel genetic syndrome with an as yet unidentified common genetic basis.

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P215

Follicle-stimulating hormone-secreting pituitary macroadenoma: a rare cause of abnormal menstrual cycles in a teenage girl

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Introduction
Gonadotroph adenomas usually present as clinically non-functioning sellar masses. They are extremely infrequent in children. Only some case report of children and adolescents with clinical manifestations of high serum gonadotrophin levels have been published. As in adult patients most of the gonadotroph adenomas have a silent growth, and therefore a late presentation as macroadenoma with mass effects.
Case report
This 16-year-old girl presented with menorrhagia, polymenorrhea and galactorrhea. Menarche had occured at 11 years of age and initially menses were regular. The patient reported a high frequent and prolonged menstrual bleeding since one year. A gynaecological examination revealed multicystic right ovary without other pathological findings and a gestagen-only pill was prescribed. After starting this treatment an oligomenorrhea with menstrual bleeding every two month occurred. Nine month later the patient developed galactorrhea and prolactin was slightly elevated with 1117 mU/l (reference $<$530 mU/l). With suspicious for prolactinoma a therapy with bromocriptin was initiated and a radiological evaluation revealed a pituitary macroadenoma (largest diameter 2.6 cm). Although prolactin levels normalized the size of pituitary mass remained unchanged. Ophthalmologic evaluation showed bitemporal hemianopsia. Medication with Parlodel® and Cerazette® was stopped and she was referred to our institution for urgent transsphenoidal resection. Preoperative endocrine evaluation showed following laboratory parameters: LH $<$0.1 IU/l (0.5–41.7), FSH 23 IU/l (1.6–17.0), estradiol 3679 pmol/l (46–1828), prolactin 2405 mU/l (78–492), IGF1 39.4 nmol/l (25.1–95.0), fT_4 16.1 pmol/l (12.6–21.0), TSH 4.0 mIU/l (0.51–4.30) and cortisol 506 nmol/l (80–638). At this time she had on-going menstrual bleeding for the last 3 weeks and no current medication. Elevated estradiol and FSH and suppressed LH was compatible with FSH-secreting pituitary adenoma. The somatotroph, thyreotroph and corticotroph axes were preserved and no disturbance of the water balance was noted. Hyperprolactinemia was interpreted as consequence of high estradiol levels and pituitary stalk compression. The postoperative course was uneventful except for a possible rhinoliquorrhea which resolved after 3 days of lumbar CFS drainage. The histopathological evaluation confirmed the diagnosis of atypical pituitary adenoma and showed immunohistochemical positivity for FSH in numerous cells. One month later normal menstrual cycle resumed and endocrine tests showed normal laboratory parameters.
Conclusion
In this case of FSH-producing pituitary adenoma, the medical history of on-going menstrual bleeding associated with highly increased estradiol, high FSH and suppressed LH were the diagnostic hallmarks. The presence of polycystic ovaries is also typical for this condition. The slightly elevated prolactin level was initially misinterpreted as evidence for prolactinoma that would have presented with much higher values in a 26 mm adenoma.

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P216

Long-term treatment with octreotide in a patient with malignant pheochromocytoma: impact on survival and time to tumor progression

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Introduction
Pheochromocytomas are rare neuroendocrine tumors arising from chromaffin cells of the adrenal medulla. Approximately 10% of all pheochromocytomas are malignant. There is no effective therapy for malignant pheochromocytoma (MAL-PHEO) and the overall prognosis is poor.
Case report
We report 22-year survival with MAL-PHEO in a patient treated with several surgeries, ^{131}I-metaiodobenzylguanidine and, subsequently, with long-acting formulation of octreotide (octreotide-LAR) for 10 years.
In 1990, a 43-year-old woman was diagnosed with pheochromocytoma. She underwent left radical nephrectomy and adrenalectomy in 1990. In 1993 and 1995, the patient underwent subsequent operations because of the tumor recurrence. In 2002, metastases in the thoracic vertebrae with a compression on the dural sac were found and the patient underwent surgical decompression in 2002 and 2004.
At referral to our Department in 2002, patient's serum chromogranin A was 342 U/l (normal range: 2–18 U/l). Somatostatin receptors scintigraphy with ^{99}mTc-EDDA/HYNIC-TOC revealed multiple vertebral and lymph nodes metastases with high expression of somatostatin receptors. In February 2003, the therapy with octreotide-LAR (20 mg i.m. every 28 days) was started. In 2004/2005 the patient was treated with ^{131}I-metaiodobenzylguanidine with a partial radiological and complete biochemical tumor response. The therapy with octreotide-LAR has been carried on from 2003 until the present time. Until 2010 radiological and biochemical stabilization of the disease had been observed. In 2010 the presence of lung metastases was revealed. Patient's serum chromogranin A increased (286 U/l in September 2012), however 24-hour urinary excretion of metanephrines is within normal range. Currently, the patient is under consideration for the treatment with radiolabeled octreotide derivates.
Conclusions
This is the first report of long-term treatment with octreotide in a patient with MAL-PHEO. Our findings suggest that, as it has been recently shown in patients with metastatic midgut neuroendocrine tumors, the treatment with octreotide-LAR may prolong survival and time to tumor progression in patients with MAL-PHEO.

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P217

Histologically surprising nasal polyps

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Pituitary macroadenomas usually present with symptoms due to a local mass effect or to hormone abnormalities. Acromegaly is due an excessive GH production, usually caused by a slow-growing pituitary adenoma. Acromegaly is an insidious disease. An average delay of 7 years is reported between the time of symptoms onset and diagnosis. According to main symptoms, acromegaly is usually diagnosed by internists, ophthalmologists or rheumatologists. It may be suspected by pneumologists in case of sleep apnea syndrome. Only exceptionally otorhinolaryngologists diagnose the disease, even if nasal polyps may be present in up to 5% of patients. We discuss here the unusual case of a 34-year-old woman who presented to an otorhinolaryngologist with persistent nasal congestion and forehead pain, unresponsive to antibiotic and anti-inflammatory treatment. Nasal endoscopy confirmed the presence of nasal polyps. Computed tomography of paranasal sinuses showed massive mucosal hypertrophy with a voluminous polypoid intranasal lesion. Transnasal biopsy demonstrated the presence of pituitary tissue. Hormonal evaluation showed a very high serum IGF-I (1185 ng/ml) and an increased serum growth hormone (48 ng/ml), leading to the diagnosis of acromegaly. The patient underwent transphenoidal surgery and histology confirmed the presence of a GH secreting adenoma. Intranasal presentations of pituitary tumours are uncommon. This case illustrates that pituitary lesions may present as polyps invading nasal cavities. To our knowledge only ten cases have been reported in literature (seven macroprolactinomas, two nonsecreting adenomas and one macroadenoma with ACTH immunopositivity). In all the reported cases initial diagnosis was that of a nasal polyp or of an intranasal carcinoma. We conclude that in a patient with nasal polyps, looking for clinical signs of Cushing syndrome or of acromegaly and subsequent hormonal evaluation could be of help for an early diagnosis.

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P218

Hypopituitarism and pituitary masses in patients with non-pituitary malignancy

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The commonest cause of acquired hypopituitarism is a benign pituitary adenoma. However, in patients with non-pituitary malignancy different diagnoses need to be considered. We describe three oncology patients presenting with hypopituitarism and/or a pituitary mass where the cause was related either to malignant disease or its treatment.

Case 1

A 56-year-old man with known metastatic melanoma presented with increasing lethargy. Investigation showed a large heterogeneous mass in the suprasellar region with a displaced normal enhancing pituitary gland. The mass was thought to be melanoma metastasis, and showed significant reduction in size (more than 70%) following treatment with vemurafenib, a B-Raf kinase inhibitor.

Case 2

A 62-year-old man with known metastatic melanoma presented with fatigue and was found to have hypopituitarism (Ft_4 – 5.0 pmol/l, TSH – 0.15 mIU/l, Cortisol – 13 nmol/l, Prolactin – 121 mIU/l, Testosterone – $<$0.4 nmol/l, LH – 1.5 IU/l, FSH – 2.5 IU/l). MRI scan of the pituitary was normal. The patient was on Ipilimumab (human monoclonal antibody directed against cytotoxic T lymphocyte antigen 4 (CTLA-4)), which has been reported to cause hypopituitarism secondary to hypophysitis (Hodi *et al. New England Journal of Medicine* **363** 711, 2010).

Case 3

A 73-year-old woman with diffuse large B cell non-Hodgkin lymphoma (DLBCL) presented with third nerve palsy and lethargy. MRI showed a large pituitary lesion thought to be either lymphoma or a pituitary macroadenoma. The pituitary lesion showed near complete shrinkage following three cycles of CHOP-R chemotherapy.

These three cases illustrate that patients with non-pituitary malignancy presenting with fatigue should be investigated for hypopituitarism. In oncology patients, the causes of hypopituitarism may include metastasis and side effects from treatment of the primary malignancy including hypophysitis.

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P219

Wegener granulomatosis as an uncommon cause of panhypopituitarism in childhood

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Wegener granulomatosis is an antineutrophil cytoplasmic antibody (cANCA)-associated, multi-system, necrotizing granulomatous vasculitis. Inflammation of nasal or oral mucosa, lung and kidney involvements are typical in the course of the disease. It was first described by Friedrich Wegener in 1939, and has an incidence rate of 1 in 100 000 people, often observed in individuals aged between 25 and 50 years, but uncommon in children and adolescent. In rare cases, pituitary involvement may occur and cause panhypopituitarism. Pituitary involvement is very rare especially in pediatric population. This is a case report of an adolescent patient who presented with panhypopituitarism symptoms and was later diagnosed with WG. Sixteen years-old female patient complained fever, headache, purulent nasal discharge and severe muscle and joint pain. Additionally she had polyuria and polydipsia. Investigations revealed pituitary mass and panhypopituitarism. The MR imaging showed a high-signal-intensity mass, which had a height of 15–19 mm, suspicious necrotic hemorrhagic regions, thickening of the pituitary stalk, hypothalamic involvement, loss of the hyperintensity of the neurohypophysis. Positivity of c-ANCA and renal biopsy result compatible with Wegener's granulomatosis confirmed the diagnosis. The patient was started on conventional treatment, which leads to the shrinking of the granuloma in the pituitary gland. A relapse did not occur over the one-year follow-up period, and the patient was still under hormone replacement therapy. WG should be considered in pediatric patients with panhypopituitarism symptoms when suspected of a granulomatous disease due to the involvement of other organs. Necrotic granulomatous lesions in MR imaging of the pituitary and ANCA positivity serve as guides in WG diagnosis.

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P220

Adequate timing for adrenalectomy in pheochromocytoma multisystem crisis: can early biochemical diagnosis be the key?

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Introduction

Pheochromocytoma multisystem crisis (PMC) is the most fulminant clinical expression of pheochromocytoma. The appropriate timing and judgment for the start of surgery and the adequate preoperative medical treatment are unclear. We report a case of PMC successfully treated with elective adrenalectomy after doxazosin blockade. The importance of an early biochemical diagnosis is discussed.

Case report

A 47-year-old healthy man consulted for sudden onset dyspnea and thoracic discomfort. In the Emergency Department, his level of consciousness upon arrival was 12 (Glasgow Coma Scale) and he was found to be in acute respiratory distress with severe hypoxemia, requiring intubation in Intensive Care Unit. His blood pressure was 165/110 mmHg and his body temperature, 39.9 °C. Electrocardiography revealed atrial flutter. The laboratory data showed polycythemia, severe renal and hepatic failure, rhabdomyolysis and hyperglycemia. All infectious screens were negative. A blood sample drawn on the patient's arrival at the hospital showed high serum norepinephrine ($>$758 pg/ml; normal $<$370). Epinephrine and dopamine concentrations were normal. An abdominal CT scan revealed a 3 cm. Mass, located in the right adrenal gland and a MIBG scan was strongly positive. After reaching clinical stability, elective right laparoscopic adrenalectomy was performed under previous α-blockade with doxazosin. Pathology examination revealed a 3 cm. Pheochromocytoma without evidence of malignant involvement, necrosis or haemorrhage. Six months after, he is asymptomatic and his level of catecholamines is normal.

Conclusions

Most reported PMC cases requiring emergency surgery or died were mixed producers of epinephrine and norepinephrine or epinephrine only. The high rate of initial suspicion allowed us an early identification of amine secreted by the tumour and it allowed us to plan a specific therapeutic strategy. Future studies with a major number of cases are needed; nevertheless our observation suggests that pure norepinephrine secretor tumours may have a more favourable clinical course and this knowledge could help to identify candidates to elective surgery after doxazosin blockade.

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P221

Complex hypothalamic disorder after childhood histiocytosis X

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Introduction

Histiocytosis X is a rare disease involving clonal proliferation of Langerhans cells, abnormal cells deriving from bone marrow and capable of migrating from skin to lymph nodes. Its manifestations range from isolated bone lesions to multisystem disease. Seen mostly in children, multifocal histiocytosis may involve in 50% of cases the pituitary stalk, leading to diabetes insipidus and

usually permanent pituitary deficiencies. We present an adult patient with a complex hypothalamic–pituitary disorder after histiocytosis X in childhood.
Case presentation
A 28 years old female has been diagnosed at the age of 4 with histiocytosis X and treated for 1 year with prednisone. She has had since diabetes insipidus treated with desmopressine and hypothyroidism treated with 50 μg thyroxine. She had grown until the age of 10 when she achieved the current height of 151 cm, her parents' heights being 170 and 180 cm. She had normal menarche with menstrual cycles of 40 days. Clinical examination revealed only slight overweight and polyuria after desmopressin withdrawal.
Hormonal evaluation (after 6 weeks thyroxine withdrawal) showed hypothyroidism FT_4 0.61 ng/dl (0.7–1.4), TSH 5.2 mUI/l (0.35–4.94), ATPO 0.45 UI/ml (<5.61), normal FSH 5.06 mUI/ml, LH 2.97 mUI/ml, PRL 19.69 ng/ml (5–26), low day-21 progesterone 0.1 ng/ml (2–24) suggesting chronic anovulation, normal basal and stimulated cortisol (16.4 and 22.9 μg/dl), severe GH deficiency IGF1 29.2 ng/ml (117–329), peak GH in insulin-induced hypoglicemia = 0.1 ng/ml. The dehydration test confirmed central diabetes insipidus. Thyroid sonography was normal. CT scan showed empty sella and pituitary hypoplasia.
Discussion
A peculiarity of this patient is the continuous linear growth for another 6 years after diagnosing histiocytosis, suggesting a late and perhaps progressive GH deficiency. In the absence of thyroid autoimmunity, the slightly increased TSH has probably low bioactivity, as described in central hypothyroidism.
Conclusion
Histiocytosis X in children may lead to a complex hypothalamic disorder persistent into adult life, including diabetes insipidus, severe (sometimes progressive) GH deficiency, hypothyroidism and chronic anovulation.

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P222

Morphologial and functional abnormalities of the hypofyse in patients with diagnose of CFS or fibromyalgia. An example of misdiagnosis by Belgian chronic fatigue centres

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Aim
In consultation, we check a lot of patients who present with diagnose of FM (fibromyalgia) and chronic fatigue syndrome (CFS). Most of these patients have a underlying diagnosis that causes chronic pain or fatigue. These causes are pathologies not easily detected.
Endocrine failure is one of the candidates, with hypofyse dysfunction as a possible candidate.
Methods
During 1 year: from October 11, 487 patients presented at the consultation we found 47 cases of morphological and functional hypofyse abnormalities. By examining with stress test in patients with clinical complaints and low basal hormones: e.g. low IGF1 or cortisol, combined with morphological abnormalities of the hypofyse.
Results
Forty seven patients with abnormalities of the hypofyse:
• Cystes: 6 cases average age 50.8, all female, mean diameter 5.2 mm (from 4 to 8 mm). All are ACTH–cortisol deficient and 1 of them is GH deficient (GHD).
• Adenomas: 31 cases average age: 42 years, 23 female, 8 males, mean size of 5 mm (from 12 to 3 mm), all are ACTH–cortisol deficient and 11 are also GHD.
• Empty cells: 12 cases: average age: 53, 25 years, 5 males, 7 females, all deficient in ACTH–cortisol and 8 are GHD.
Conclusion
Patients with a diagnose of CFS or fibromyalgia should always be checked for underlying chronic diseases. Mostly immunologic but also endocrine diseases can be underlying. E.g. frequently adrenal insufficiency can be detected. A lot of reports document also a low IGF1 and GHD.
Patients with hormone deficiencie should also be checked for other hormone deficiencies. In case of low hypofyse hormones, single or multiple, the hypofyse has to be functionally and morphologically checked. On contrary with the disappointing general therapy of FM or CFS, a good and efficient therapy can be offered to patients by treating the underlying hormonal deficiencies.

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P223

Pineal gland tumor and panhypopituitarism in an adult male

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Background
Pineal germ cell tumors are rare in adulthood. It usually comes to medical attention due to mass effect. Endocrine manifestations are also prominent in the clinical presentation and may precede neurologic symptoms.
Clinical case
We report the case of a 30-year old male who presented with a 6-month history of bitemporal headache, doubling of vision and nonprojectile vomiting. Recall revealed that he exhibited symptoms of panhypopituitarism as early as two years prior. He also had excessive thirst, polyuria and nocturia. Five months upon presentation, he developed rapidly progressive pain then numbness of both lower extremities which eventually culminated in loss of motor strength and sensation. Physical examination showed limitation of vertical movements of both eyes, paraplegia and hypoesthesia of both lower extremities.
Investigations confirmed anterior pituitary failure with central hypothyroidism, adrenal insufficiency, hypogonadism and diabetes insipidus. Tumor markers alpha-fetoprotein and beta HCG were elevated. Imaging revealed obstructive hydrocephalus and a $5.8 \times 5.4 \times 4.6$ cm pineal gland tumor with normal sella. Magnetic resonance imaging of the spine showed spinal cord lesions and leptomeningeal enhancement. A diagnosis of pineal nongerminomatous germ cell tumor with spinal cord and leptomeningeal carcinomatosis was made.
Hormonal deficiencies were replaced with oral L-thyroxine, prednisone and desmopressin. The patient underwent cranial irradiation which resulted in more than 50% regression in the tumor size. Spinal radiotherapy yielded sensory but no motor recovery. Chemotherapy was initiated using bleomycin, etoposide and cisplatin. He developed nosocomial pneumonia and febrile pneumonia 1 week later to which he succumbed.
Conclusion
The characteristic clinical triad of diabetes insipidus, anterior pituitary failure and visual disturbances is a harbinger of a pineal germ cell tumor. It may present with panhypopituitarism even in the absence of pituitary involvement on imaging. Thorough endocrine evaluation and hormone replacement is essential in its management.

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P224

Antepartum pituitary insufficiency in type 1 diabetes

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Introduction
Postpartum necrosis of the anterior pituitary gland is well-described, but antepartum pituitary insufficiency (API) is reported only in a few women with type 1 diabetes (T1D). API has an abrupt onset and may have fatal outcome.
Case report
A 36-year-old woman with T1D and laser-treated retinopathy became pregnant after repeated IVF. In gestational week (GW) 10, she used insulin 34–42 U/day, HbA1c was 6.2% and cortisol (0800 h) was 664 nmol/l. In GW 34, she was hospitalized with intense headache and vomiting. On admission, plasma glucose was 2.6 mmol/l. Neurological examination, CSF and cerebral MRI, were normal. She had no sign of preeclampsia. Insulin doses were reduced. At 0800 h. cortisol was 144 nmol/l, ACTH <1.1 pmol/l, prolactin 285 mU/l, GH 1.5 mU/l, indicating pituitary failure. She was treated with i.v. glucose and hydrocortisone. An emergency Caesarean section was performed, without excessive haemorrhage. She was nomotensive. The newborn's weight was 2610 g and Apgar score 9 (1 min). Three days *postpartum* (PP) pituitary MRI was normal. She was unable to breastfeed. Two months PP, insulin tolerance test (ITT) showed subnormal cortisol and ACTH responses (436 nmol/l and 2.8 pmol/l, respectively). Despite substantial reduction in insulin dose and low-dose cortisol replacement, she experienced frequent hypoglycaemias without warning. Repeated MRI at three

months PP revealed reduced contrast uptake in the pituitary consistent with necrosis. ITT after 2 years showed subnormal responses of both cortisol (peak 468 nmol/l) and GH (peak 4.5 mU/l). GH replacement improved her hypoglycaemia awareness and her general well-being.

Conclusion

The hypertrophied pituitary of late pregnancy may be vulnerable to infarction and necrosis. We speculate whether repeated ovarian stimulation together with microvascular diabetic complications may have increased the vulnerability to thromboembolism and subsequent pituitary infarction in this woman with API.

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P225

Delivery of health child in acromegaly patient with McCune–Albright syndrome treated with lanreotide and pegvisomant during pregnancy

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Introduction

Acromegaly with GH excess affects up to 20% of the patients with McCune–Albright syndrome (MAS). Surgical treatment for acromegaly in MAS is often difficult because of skull-base dysplasia. Somatostatin analogs are frequently only partially effective and GH receptor antagonist – pegvisomant is more potent in normalizing IGFI levels. Radiotherapy is controversial. Pregnancy in MAS patients is described in literature but no case of successful delivery in MAS patient with acromegaly treated with both somatostatin analog and pegvisomant was published till now. Only three cases of pegvisomant treated pregnant acromegalic patients have been published (two with medication throughout the whole pregnancy).

Case report

At the age of 32, diagnosis of MAS with multiple endocrine disorders (acromegaly, peripheral hyperthyroidism, hyperparathyroidism) was diagnosed. Neurosurgery and/or irradiation was not possible due to serious osteodysplasia in sellar region and medicament treatment was started using long-acting somatostatin analogue lanreotide, which was later combined with GH antagonist pegvisomant. At the age of 36, woman became pregnant. The patient insisted on pregnancy. We continued lanreotide and pegvisomant treatment. Pregnancy and delivery was not complicated and healthy girl was in 38th week of gestation born (cesarean section). Lactation was blocked. Further development of this girl is normal. We continue with lanreotide and pegvisomant treatment.

Conclusion

We report successful pregnancy in an acromegalic patient with MAS treated by combination of pegvisomant and lanreotide. Child's weight and length was lower, influence of therapy by lanreotide and pegvisomant can be discussed. Father's body constitution could have some influence as well. No congenital malformations were present, development is physiological, there are no signs of endocrine/bone disorders.

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P226

Intrasellar plasmacytoma: an unusual presentation of multiple myeloma

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Multiple myeloma is a neoplastic disorder arising from plasma cells in the bone marrow. Plasma cell tumors, plasmacytomas, are uncommon in the brain and occur usually in the leptomeninges with or without parencymal involvement. Extramedullary plasmacytomas are very rarely located in the sella and can be misdiagnosed as a nonfunctioning pituitary adenoma. We report an illustrative case with multiple myeloma who was diagnosed having sellar plasmacytoma resembling a nonfunctioning pituitary adenoma.

A 56-year-old Caucasion woman presented with a history of headache and diplopia. She denied any other neurological symptoms. Her complaints started approximately 6 months ago. She had a mild anemia with a hemoglobin level of 11.1 g/dl (12–14 g/dl). Blood chemistry analyses were normal. Serum prolactin level was slightly elevated at 76.44 ng/ml (1.2–29 ng/ml), but other hypophsial hormones were within normal limits. Magnetic resonance imaging (MRI) of the brain showed a $26 \times 22 \times 31$ mm mass destroying the floor of sella and infiltrating the clivus. The presumptive diagnosis was of a nonfunctioning pituitary adenoma and transsphenoidal gross excision was performed. The histological appearance of the specimen was that of a highly cellular neoplasm composed of mature plasma cells with round eccentrically located nuclei. Immuno-cytochemistry showed that all the tumor cells were positive for CD 138 and lambda light chains confirming that this tumor was a plasmacytoma. Post operatively an extensive investigation for myelomatous disease was undertaken including serum and urine protein electrophoresis, a bone survey and a bone marrow biopsy. Serum protein electrophoresis detected a monoclonal gammophathy with IgG kappa type M protein on serum immunfluorescence electrophoresis. Urine was negative for Bence-Jones proteinuria. Beta 2 microglobulin level was 3.2 mg/dl. Bone marrow biopsy showed plasma cell infiltration. The diagnosis of multiple myeloma with involvement of the pituitary gland was made. Subsequently, systemic chemotherapy consisting of melphalan and prednisone was given for 6 cycles. The patient remained in remission for 20 months after the therapy.

In conclusion, although multiple myeloma involving pituitary gland is rare, it must be considered in the differential diagnosis of a nonfunctioning pituitary mass. A hypophysial mass with a normal anterior pituitary hormonal profile should alert the physician for the possibility of a diagnosis other than a chromophobe adenoma.

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P227

Corticomedullary mixed adrenal tumor

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Introduction

Adrenal glands consist of two parts: adrenal cortex and adrenal medulla acting as two separate organs due to distinct structure, function and embryologic origin. Corticomedullary mixed tumor is an adrenal tumor mass which consists of mixed population of both adrenal cortical cells and medullar chromaffin cells.

Case report

A 36-year-old woman was admitted to our Clinic with hypertensive episode, headache and palpitation. Ultrasound and CT confirmed right adrenal tumor, size of 55 mm. Active pheochromocythoma was confirmed by elevated urinary catecholamines and serum chromogranine A. Endocrine evaluations revealed normal midnight cortisol, basal ACTH with adequate cortisol suppression after 1 mg-DST. PRA and aldosteron were also in normal range with normal ALD/PRA ratio. After careful administration of alpha-blockers (phenoxybenzamine) surgery was performed. Pathohistology and immunohistochemistry of the tumor showed mixed corticomedullary tumor with element of pheohromocythoma and adrenocortical adenoma. Adrenocortical cells expressed melanin A, inhibin, calretinin, and synaptophysin and medullar component expressed chromogranin A and synaptophysin with a few sustentacular S100 positive cells. Postoperatively, urinary catecholamines and serum chromogranine A were in normal range.

Conclusion

We presented a case of pheochromocythoma in a patient with confirmed corticomedullary mixed adrenal tumor which are quite rare. Clinical cases like this one underline the significance of thorough endocrine evaluation of every patient with adrenal tumor. The tumorigenesis of these tumors remains open.

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P228

Xanthomatous hypophysitis as a cause of cluster headache: a case report

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Introduction
Hypophysitis is an inflammatory disease of the pituitary gland that may mimic pituitary tumors clinically and radiologically. Primary hypophysitis has traditionnaly been classified as lymphocytic (LH), granulomatous (GH), and xanthomatous (XH).
Case description
We report on a case of a xanthomatous hypophysitis initially diagnosed as pituitary adenoma. A 23-year-old men suffered from typical cluster type headache. Two years after the first symptoms, we confirmed diabetes insipidus in the background of polyuria–polydipsia. All the anterior pituitary hormone levels were normal. Head MRI scan depicted a $14\times10\times17$ mm inhomogenous mass int he sella. Transphenoidal surgery was performed; the removed tissue showed by accumulation of foamy cells and *xanthomatous epithelioid cells*. The patient had an uneventful postoperative recovery. After surgery, we tried to stop the hydrocortisone therapy but severe cluster type headache returned. The endrocrine work up revealed hypadrenia (morning cortisol: 96 nm/l, ACT: 3.38 pm/l), hypothyroidism (ft_4: 10.5 pm/l), hypogonadism (testosterone: 3.44 nm/l) with normal FSH and LH (FSH: 3.3 mIU/l, LH: 2.8 mIU/l). We restarted hormonal therapy: hydrocortisone, L-thyroxine and testosterone were stepwise reintroduced. During the follow-up period we could stop the hydrocortisone and L-thyroxine supplementations, whereas the patient has permanently required desmopressin and testosterone substitution. Because of his hypogonadism after pituitary surgery, the patient was sent for sperm storage. Control sella MRI scans revealed no progression of the intitially seen pitiutary tumor.
Conclusion
We describe an unusual case of xanthomatous hypophysitis causing cluster type headache and permanenty requiring ddAVP (desmopressine) and testostreone supplementatation however, without need for maintenance medication with hydrocortisone and L-thyroxine.

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P229

Primary adrenal insufficiency in a case of bilateral adrenal hemorrhage secondary to anti-phospholipid syndrome masquerading as chronic abdominal pain

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Background
Primary adrenal insufficiency due to adrenal hemorrhage from anti-phospholipid syndrome (APS) is an uncommon and life threatening disorder. We present a case, highlighting the challenges in the diagnosis and management of this disorder.
Case presentation
A 59-year-old Chinese lady presented with a 2-year history of bilateral flank pain, lethargy, poor appetite and weight loss. Initial blood investigations were unremarkable except for a cholestatic liver function test. A computed tomography of the abdomen incidentally revealed bilateral adrenal hemorrhages. She had not sustained any trauma, did not have a bleeding disorder and was not taking any anticoagulants. Subsequent workup confirmed the diagnosis of primary APS with elevated levels of lupus anticoagulant, anticardiolipin and anti-B2 glycoprotein. She did not have any other associated connective tissue disorders or malignancies. Thrombophilia screen was normal. There was concomitant primary adrenal insufficiency with a blunted synacthen test response. Cortisol was 35 nmol/l at 0 min and 34 nmol/l 30 min after synacthen was given; ACTH was markedly elevated at 194 pmol/l. Aldosterone was undetectable while renin was elevated at 4.96 ng/ml per hour. She was started on hydrocortisone and fludrocortisone in addition to anticoagulation. Interval adrenal imaging showed near complete resolution of adrenal hemorrhage but the adrenal insufficiency persists with blunted synacthen test and undetectable aldosterone despite 1 year of follow up.
Conclusion
Clinicians need to have a heightened awareness of the association between adrenal hemorrhage and APS and institute treatment early. Despite radiological resolution of the adrenal hemorrhage, adrenal insufficiency persists, likely secondary to irreversible hemorrhagic infarction of adrenal tissue.

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P230

Very late growth acceleration in a man with hypopituitarism

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Introduction
Very late step growth is quite rare, and probably very rare, if there is, 10 years after the end of treatment with GH. Therefore, we would like to present our patient, even though we cannot yet give a full pathomechanism of his disorders.
Case report
A man 28 years, was admitted to us to assess hormonal status before elective hip surgery. He had congenital brain toxoplasmosis. From 8 to 16 years he was treated with GH without a good effect. However, in the last 2 years, so between 26 and twenty eight years, he has grown more than 20 cm. Laboratory results indicated hypopituitarism with undetectable GH and low IGF1, adrenal insufficiency (cortisol 18 μg/l, ACTH 21 ng/l), and hypothyroidism (FT_4 7.2 pmol/l and TSH 5.8 mIU/l) and hypogonadism with undetectable testosterone levels and LH <0.20 mIU/l, FSH 0.4 mIU/l and estradiol 29 pg/ml. In inguinal canal and scrotum, we found small fragments of glandular tissue. The response to gonadothropin was week. In MRI normal pituitary $5\times6\times9$ mm, and at the bottom of third ventricle 5 mm size, expansive tissue. No signs of puberty, height 174 cm, weight 54 kg BMI 17.8. The epiphyses are not fused together and the patient is still growing despite the very low IGF.
Adrenal replacement therapy was started followed by thyroid replacement therapy. We consider the operations to remove the glandular tissue of the inguinal canal and scrotum and substitutions with testosterone.
Discussion
We take into account the possibility of ectopic secretion of IGF other than growth factors and/or factor causing sensitivity to IGF. However, this remains speculation. Therefore, we would like to present him hoping for help from the European Endocrinologists.

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P231

Differential diagnosis of aggresive macroprolactinoma, adenoma or atypical adenoma: a case report

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Atypical tumors were identified in 15% of pituitary adenomas, and they tended to be aggressive, invasive macroadenomas. WHO classification of atypical pituitary adenomas include; Ki-67 proliferative index $>3\%$, excessive p53 immunoreactivity, and $\geq$ 2 mitotic figures per 10 high-powered fields. Pituitary carcinomas are extremely rare tumors with cerebrospinal or extracranial metastasis.
Case
A 31 years old man with symptoms of stuffy nose and snore, presented to our policlinic due to the solid lesion on paranasal sinus tomography. Tomography showed a soft tissue lesion with 38 Haunsfield Unit (HU). Pituitary imaging revealed a mass, which lead to destruction of bone structures, suppression of optic chiasm, extending to suprasellar cistern and right nasal cavity. Except increased prolactin (470 ng/ml) hormonal levels were in normal ranges. Macroprolactin was negative. Nasal punch biopsy showed an atypical pituitary adenoma with atypical and 8 mitotic figures in the 10 high-powered fields. Ki-67 labeling index 2–3%, p53 immunoreactivity was 1%. 18-Fluoro-Deoxy-Glucose Positron Emission Tomography determined increased activity in this lesion with 6.4 SUV-max. Distant metastasis was not determined. Cabergolin 0.5 mg/ twice a week was begun and then he underwent to operation. Histopathological result was a pituitary adenoma with diffuse staining PRL. Ki-67 index was 1%, whereas p53

immunoreactivity 10%. Postoperative PRL level reduced to 65 ng/ml and no solid lesion was seen in postoperative imagining. His medical treatment is now ongoing.
Conclusion
Because of the atypical and ≥ 2 mitotic activity, necrosis in the nasal punch biopsy, also 38 HU solid lesion in tomography and absent of distant metastasis, we thought primarily atypical adenoma in this case. Although histopathological result is consistent with benign prolactinoma, it must be considered that its biological behavior may be progressed to malignancy after several years.

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P232

Bilateral third nerve palsy secondary to an apoplexy in a pituitary macroadenoma causing Cushing's disease: a very rare complication of a rare entity

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Introduction
Bilateral 3rd nerve palsy is known in conditions such as diabetes mellitus, neurosarcoidosis, Guillain-Barre syndrome, multiple sclerosis, anterior or posterior communicating artery aneurysm or mesencephalic bleed/trauma. There are only single cases reported in association with pituitary adenoma or carcinoma, usually in the context of apoplexy. We describe a patient with Cushing's disease and bilateral 3rd nerve palsy secondary to apoplexy in pituitary macroadenoma.
Case presentation
A 54-year-old man with background of ulcerative colitis, recent-onset hypertension, hypokalaemia and type 2 diabetes was referred to the gastroenterologist with weight loss. CT of the abdomen revealed bilateral adrenal enlargement and a random cortisol was elevated at 1800 nmol/l, and he was referred to the endocrine unit. The patient was admitted for further investigation: he had a severe proximal myopathy and peripheral oedema. His midnight sleeping cortisol was 3200 nmol/l (normal <50 nmol/l). As his ACTH was elevated at 188 ng/l, a MRI scan of the pituitary was arranged and showed a pituitary macroadenoma with left cavernous sinus invasion. The patient rapidly developed a left-sided 3rd nerve palsy, which was followed by confusion and then right 3rd nerve palsy 24 h later. His serum cortisol increased to 7500 nmol/l. A CT confirmed haemorrhage into the pituitary macroadenoma. The patient developed septicaemia and associated thrombocytopenia and was not fit for transsphenoidal surgery. Four days following the apoplexy his 0900 h serum cortisol fell to 270 nmol/l and in view of his sepsis hydrocortisone replacement was added. Six weeks later patient remains hospitalised with persistent bilateral 3rd nerve palsy and is awaiting pituitary surgery after rehabilitation.
Conclusions
Bilateral 3rd nerve palsy, though very rare, can occur in Cushing's disease, and if of acute onset is suggestive of pituitary tumour apoplexy. In this patient, the massive surge in serum cortisol was considered to be secondary to the pituitary apoplexy.

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P233

Good response to temozolamide therapy in a man with a prolactin secreting pituitary carcinoma

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Introduction
Pituitary carcinomas are rare and their management is difficult, because they exhibit a continued growth and metastatic dissemination despite of multimodal and aggressive treatments. Temozolamide, has shown a substantial response rates in pituitary carcinomas.
We report the case of a patient with a malignant prolactinoma successfully treated with temozolamide.
Case report
The patient, a 62-year-old male, consulted with severe headaches. He was diagnosed of a macroprolactinoma but due to poor response to cabergoline and persistence of the headaches, he underwent surgery in 2 ocassions, and was treated with conventional radiotheraphy and cabergoline which allowed control of prolactin, headaches and pituitary remant for a year. By the time that the headaches were back, the prolactin levels showed lack of response to cabergoline and bromocriptine treatment, and he developed hepatic, bronquial and vertebral metastasis.
He was commenced of Temozolamide (200 mg/m^2, 5 days every 28 days) for 18 cycles and the prolactin levels responded dramatically, as well as the residual tumor size and the hepatic and bronquial metastasis. He received radiotherapy for the vertebral metastasis and is currently still receiving temozolamide. The treatment has been well tolerated.
Conclusion
Although pituitary carcinoma remains difficult to diagnose and manage, it seems that Temozolamide, an orally administered alkylating agent, may be an effective option for pituitary carcinomas.

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P234

Combined choroidal neovascularization and hypopituitarism in a patient with homozygous mutation in methylenetetrahydrofolate reductase gene

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Introduction
Hypopituitarism is defined as either partial or complete deficiency of anterior or posterior pituitary hormone secretion or both. Hypopituitarism itself may increase the risk of thromboembolism/hypercoagulopathy, and underlying mechanisms of hemostatic dysfunctions in hypopituitarism are mostly unknown. Reduced enzymatic activity due to methylenetetrahydrofolate reductase (MTHFR) gene mutations are associated with hyperhomocysteinemia and have been linked to both arterial and venous thrombosis. Choroidal neovascularization (CNV) in the macular area is one of the major causes of severe visual. Relationship between MTHFR and hypopituitarism in patients with Sheehan syndrome was also shown previously. Except for the Sheehan syndrome, any association of MTHFR mutation with hypopituitarism could not be identified to date.
Case report
We report a case of choroidal neovascularization (CNV) secondary to MTHFR gene mutation in a 20-year-old male patient with hypopituitarism. There was no history of pituitary surgery, radiotherapy, cranial trauma, pituitary apoplexy, subarachnoid hemorrhage or ischemic stroke. Finally, no primary cause of hypopituitarism could be found except a MTHFR gene mutation. Treatment with three consecutive injections of intravitreal ranibizumab (anti-vascular endothelial growth factor) resulted significant improvement of the patient's vision and the appearance of the macula. With hormone replacement therapy of hypopituitarism also acetyl salicylic acid 100 mg/day was started. The patient was clinically stable both for CNV and other thromboembolic disorders over a 1-year follow-up period.
Conclusion
CNV and hypopituitarism associated with a MTHFR gene mutation is highly unusual. Although there are no recommendations in this regard, the observations in the present patient indicate that antiangiogenic therapy can be useful and safe for the treatment of CNV in such a condition. Along with corticosteroids, levotroxin and testosterone replacement for the hypopituitarism, ASA treatment to prevent recurrent embolic events could be a reasonable approach when thrombotic ophthalmic complications occur in subjects with a MTHFR gene mutation.

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P235

Acute pulmonary edema as the initial presentation of a pheochromocytoma: case report

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Introduction
The acute onset of pulmonary edema and severe congestive heart failure secondary to catecholamine overproduction from a pheochromocytoma is a rare

entity, especially in the absence of other signs or symptoms suspicious of this tumour. Myocardial involvement can include angina pectoris, acute heart failure, dilated cardiomyopathy, myocardial infartion and arrhytmias. Here, we present the case of a young man with no significant medical history who presented with acute pulmonary edema and dilated cardiomyophaty secondary to a unknown pheochromocytoma.
Case report
A 29-year-old caucasian male presented in the emergency room with an acute pulmonary edema, which resolved with minimal therapy; he had no cardiovascular risk factors (including hypertension), but he complained of palpitations and diaphoresis since 3 months ago. It was performed an echocardiogram which revealed a dilated cardiomyopathy with severe decline of systolic function. Twenty-four hour catecholamine and metanephrine levels were obtained: total metanephrine level of 6073 μg/24 h (normal value $<$1600), normetanephrine of 5197 μg/24 h (normal between 105 and 354), total catecholamine level of 1281 μg/24 h (217–575), noradrenaline of 958 μg/24 h (23–105). He was ordered an abdominal computed tomography, which revealed a 4.8$\times$3.7 cm left adrenal mass. The patient was started on phenoxybenzamine (10 mg by mouth four times a day), and posteriorly with carvedilol (6.25 mg twice a day); 2 months later he underwent a laparoscopic left adrenalectomy with no complications. In the follow-up, it was verified a complete regression of the cardiomyopathy, with return to normal systolic function; he is currently doing well.
Discussion/Conclusion
Pheochromocytoma is a rare clinical entity and a diagnostic challenge, even more in this clinical setting; it should be included in the differential diagnosis of acute congestive heart failure when no other obvious cause can be elicited.

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P236

Pleuropericarditis effusion secondary to chronic bromocriptine intake

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Introduction
Dopamine agonists are commonly used for the treatment of Parkison's disease and prolactinomas. Sometimes high doses are needed in mixed pituitary adenomas. This medical treatment usually induces gastrointestinal troubles and/or low blood pressure, and sometimes psychiatric disorders. Pleuropercarditis effusion (PPE) is rarely related to dopamine agonist side effects.
Case report
Our aim is to report a man aged 29 years old whose PPE is apparently due to high dose and chronic bromocriptine intake.
This person, followed for 4 years for a somato-lacto-thyreotrop pituitary macro adenoma, was first treated by a combination of anti thyroid drugs, somatostatin's analogs and bromocriptine (12.5 mg/day). After normal thyroid function achievement, he was operated via transphenoidal approach. Unfortunately, pituitary surgery was totally unsuccessful, as he had only a biopsy. This situation obliged us to prescribed bromocriptine again with a higher dose (25 mg/day). Eighteen months later, this man had an acute respiratory distress with orthopnea and muffling of heart sounds without fever or alteration of his general condition. Chest X-rays revealed a mild right pleural effusion with normal lung parenchyma. Echocardiography showed abundant pericarditis effusion that required paracentesis. Abdominal ultrasound did not find peritoneal effusion.
Cardiovascular, hepatic and renal causes were excluded. Research for neoplasms and tuberculosis were negative. Protein electrophoresis and thyroid function were normal too. As we did not have any aetiology, the iatrogenic cause seemed more probable, so bromocriptine was stopped. For more than 2 years, we did not notice any PPE relapsing which reinforced our supposition. The mechanism of PPE secondary to ergocriptine intake is probably immuno-allergic.
Conclusion
PPE secondary to bromocriptine intake is an exceptional adverse effect that should be kept in mind after exclusion of PPE's classical aetiologies. Periodic echocardiography and chest X-ray should be done in patients under long-term bromocriptine therapy.

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P237

Suppurative meningitis as a life threatening primary presentation of macroprolactinomas

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Introduction
Suppurative meningitis (SM) is a life threatening disease. It is rarely observed as a primary presentation in large pituitary tumours (PT) destroying the sellar floor and/or invading the skull base. Our aim is to report 3 SM revealing macroprolactinomas.
Case No. 1
A man aged 22, consulted for vomiting and fever. The diagnosis of SM was confirmed by lumbar puncture and blood cultures. Cerebral MRI showed multidirectional PT invading cavernous sinuses. Hormonal assessment demonstrated high prolactin with pituitary deficits (PD). After antibiotics SM was sterilized. Then, the PT was treated with dopamine agonists that were successful on prolactin and tumour size. The SM never relapsed, although the sellar floor was not surgically repaired.
Case No. 2
A male aged 49 hospitalized for the fourth bacterial SM episodes. Cerebral MRI showed an invasive and multi directional PT. The SM was cured by antibiotics. Prolactin was normalized by bromocriptine and the tumour size was significantly reduced. There was not any SM relapse.
Case No. 3
A man aged 25, with a history of arrested puberty, diagnosed as having a macroprolactinoma, with supra, infra and latero-sellar expansions revealed by a SM due to pneumococcal infection. The SM was sensitive to antibiotics, prolactin normalized under bromocriptine and tumour volume decreased progressively. There was not any relapsing.
Conclusion
Primary SM (PSM) rarely reveals PT, except if this one is very aggressive. To our knowledge only 52 cases have been reported. PSM can precede cerebrospinal leak which should be treated by surgery to avoid life threatening complications.

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P238

Craniofacial fibrous dysplasia and pituitary gigantism in a 10-year-old boy: clinical case

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Introduction
The combination of poly/monostotis fibrous dysplasia, café-au-lait pigmentation of the skin and endocrine hyperfunction (mostly precocious puberty) is known as McCune–Albright syndrome (MAS), a genetic origin syndrome with low incidence. The molecular basis of MAS is a mosaic activating mutation of the α subunit of the G protein (Gsα) gene.
We present a clinical case of a 10-year-old boy with partial MAS and GH-secreting pituitary adenoma treated with octreotide-LAR.
Clinical case
The patient at the age of 5 years manifested headache and growth acceleration (10 cm per year). At 10 years, he was 176.6 cm tall. Examination revealed elevated serum GH (20 ng/ml, supression during OGTT minimum to 11.3 ng/ml), IGF1 (1176 ng/ml), IGF-BP3 (362 nmol/l) and prolactin (2480 IU/l). MRI found a pituitary microadenoma and CT showed signs of fibrous dysplasia of frontal, sphenoid and ethmoid bones. No café-au-lait spots or other endocrinopathy was observed. The therapy with octreotide-LAR and cabergoline was started. During 4 years of follow-up patient developed normal puberty and grew up on 27 cm. Lab tests showed normalisation of prolactin (with no elevation during 2-year self-withdrawal period) and appearance of secondary hypothyroidism. He receives octreotide-LAR 40 mg for at least 2 past years and cabergoline 0.25–1 mg per week. The decrease of IGF1 (to 700–800 ng/ml which is within the 95th percentile) and GH (minimum to 7.3 ng/ml) were observed. In 4 years of treatment, IGF1 level rose again to 1182 ng/ml. No CT and MR signs of tumor progression/reduction or deterioration of bone lesions were seen.
Conclusion
We report a clinical case of MAS in a child associated with GH-secreting adenoma treated conservatively with octreotide-LAR. The therapeutic response was insufficient and curative treatment was required. Despite there is a partial

MAS with few organ involvement, the specifity of endocrinopathy determines a burden of the disease.

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P239

Functional and transient effect of sodium excretion in combined pituitary failure with central and peripheric diabetes insipidus

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Introduction
Central and Peripheral Diabetes Insipidus are both rare conditions. Combined they may result in serious hypernatremia and water deficit that may pose a therapeutic challenge.
Case report
MHBCR, a caucasian female aged 52, was admitted to the Endocrine Department because of serious hypernatremia. A previous diagnosis of pituitary failure and central diabetes insipidus was established 4 years before, after pituitary surgery for a non-secreting pituitary adenoma and was treated with hydrocortisone 20+5+5 mg/day, L-thyroxine 125 μg/day and desmopressin 60 μg/day po and 80 μg/day intranasal. Because of an acute psychotic episode she was medicated with lithium 400 mg/day, 7 days before. On admission hypernatremia was found (153 mEq/l), with normal serum potassium and renal function. Urine output was 12 400 ml/day. Litium was interrupted, fluid i.v. administration was increased up to 6000 ml/day (normal and hypotonic saline), desmopressin 4 μg, i.v. 12/12 h, hydroclorothiazide 50 mg/day and indometacin 400 mg/day were initiated. In the next few days, urine output decreased to 6–7 l, water balance was almost null, with normal urea, but serum sodium increased to 180 mEq/day. Desmopressin was increased up to 4 μg, i.v. 4/4 h but was ineffective. Dialysis was begun at the seventh intra-hospital day with correction of serum sodium to 160 mEq/l in a single session. Furosemide 40 mg/day, i.v. further normalized serum sodium to 132 mEq/l in the next few days.
Discussion
Combined central and peripheral diabetes insipidus may result in serious life-threatening hypernatremia because of massive water loss. In this case both conditions were conventionally treated but despite no evidence of hemoconcentration, sodium further increased in a dramatic way, pointing to a specific sodium excretion defect. The most likely explanation is increased renin–angiotensin–aldosterone activity because of volume depletion with increased distal tubule reabsorption of sodium. This possibility is supported by the transient nature of the defect and the very good response to furosemide.

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P240

Management of resistant prolactinoma: case report

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Introduction
Prolactinomas are the most common tumors among hormonally active pituitary adenomas. Therapy with dopamine agonists (DA) remains the first treatment choice. However there remain definite numbers of prolactinomas resistant to standard DA therapy. The underlying mechanisms of this phenomenon, management and prognosis are still poorly understood. The search of treatment options for overcoming DA-resistance is an important object in practical endocrinology. We present a clinical case of successful treatment of a man with partially DA resistance with co-administration of quinagolide and cabergolin.
Clinical case
A 58-year-old man presented slight visual impairment, headache and fatigability. Magnetic resonance imaging (MRI) found a endo-infra-suprasellar pituitary adenoma. Laboratory examination revealed hyperprolactinemia (prolactin 4961 IU/l) and secondary hypogonadism. Administration of dopamine agonists (max dose of Cabergolin 5.5 mg per week) led to improvement of visual function and slight decrease of prolactin level (not normalization) with no effect on tumor size on MRI evidence. The decision to add Quinagolide (in max dose 150 μg daily) to therapy was made after 2.5 years of Cabergolin monotherapy. Cabergoline dose was slightly diminished. After 5 months of the combine therapy was observed normalization of prolactin and testosterone level.
Conclusion
This clinical case is an example of successful treatment of partially DA-resistant prolactinoma. The combination of different dopamine agonists allowed to overcome medication resistance and restore gonadal function.

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P241

Addisonian crisis: rapid correction of hyponatriemia leads to osmotic myelinolysis

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Chronic hypovolemic hyponatriemia is typical for Addisonian crisis and may provoke brain edema but its rapid correction may lead to myelinolysis. We report 2 newly diagnosed cases of primary adrenal insufficiency (PAI) associated with severe hyponatriemia which correction was complicated by osmotic myelinolysis.
Case 1
Twenty four-year-old female was admitted with vomiting, sopor, shock. Serum sodium was 83, glucosae 3.6 mmol/l. Serum cortisol 34 nmol/l and ACTH 307 pmol/l confirmed PAI. Hydrocortisone and saline were administered. Within 2 h – apnoe, mechanical ventilation began, than coma revealed. 3% saline infusion was added: 400 ml during 4 h giving sodium rise to 107 mmol/l. Saline infusion continued and within 24 h serum sodium was 117 mmol/l (37 mmol/l rise per day). Magnetic resonance imaging (MRI) didn't reveal the reason for persisting coma. Diagnosis of central pontine myelinolysis was considered and was confirmed later by MRI. Patient dies after 18 months lasting coma.
Case 2
Fifty four years female was admitted with abdominal pain, vomiting, hypotension, hyperpigmentation. Serum sodium was 104, glucose 4.6 mmol/l, cortisol 190 nmol/l; plasma ACTH 246 pmol/l, renin 40 ng/ml. Saline infusion begins and additionally 450 ml 3% saline during 10 h was administered giving sodium rise to 116 mmol/l. Than saline was infused and to the next day sodium level was 122 mmol/l (increase −18 mmol/24 h). After 3–4 days of compensation parkinsonian signs appeared. MRI revealed extrapontine myelinolysis. After 3 months of treatment – full neurological recover.
These cases highlight the importance of careful correction of hyponatriemia in PAI. Fatal pontine myelinolysis in case 1 developed due to overzealous correction of chronic hyponatriemia without necessary control and not in line with current recommendation: possible rate of correction is 8 mmol/l during the first and 8–10 during second days and only at first hours 1–1.5 mmol/l per hour increase is possible.

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P242

Pituitary apoplexy after suppression test in a patient with Cushing's disease due to macroadenoma

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Introduction
Cushing's disease is not likely to be pituitary apoplexy or macroadenoma. We present a case of pituitary apoplexy after dexamethasone suppression test for Cushing's disease due to macroadenoma.
Case report
A 30-year-old man complained of headache and closure of the left upper eyelid. Two years ago he had a headache, and was diagnosed with non-functional macroadenoma of pituitary. In follow, insuppressibly serum levels of cortisol with dexamethasone were detected with normal levels of ACTH. He complained with headache and ptosis in the left upper eyelid with sudden loss of vision in the left. Magnetic resonance imaging of the sella turcica showed definitive pituitary apoplexy and macroadenoma. Hemorrhagic diathesis tests were normal. He was treated successfully by endoscopic endonasal transsphenoidal surgery within several hours after onset of pituitary apoplexy. His symptoms and signs were significantly improved.
He is followed by glucocorticoid replacement therapy with L-thyroxine and androgen.
Conclusions
Endocrine stimulation tests need to be careful in patients with pituitary macroadenoma, and should be assessed on a case-by-case basis. Pituitary apoplexy and macroadenoma in the present patient is not likely to be seen in Cushing's disease.

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P243

Osteoporotic fractures as manifestation of Cushing's disease

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Introduction
Cushing's syndrome (CS) is a well-known cause of bone loss and osteoporotic fractures, which may be the initial manifestation of the disease and may present 2 years before CS diagnosis. Trabecular bone is usually the most seriously affected, and vertebrae and ribs are the typical fracture locations.
Case report
A 37-year-old man with dyslipidemia and obesity was observed due to uncontrolled hypertension and osteoporosis. He complained of back pain and loss of 6 cm height in the last year. Dorsal and lumbar spine X-ray performed before the appointment showed D12 vertebral body fracture and bone densitometry revealed osteoporosis. Physical examination revealed BMI 29.4 kg/m^2, mild facial plethora, thin limbs and central obesity. The hormonal study showed urinary free cortisol 687.3 μg/24 h (36–137) and ACTH 118 ng/l ($n<53$). Thyroid function and 24-h urine catecholamines and metanephrines were normal. Serum cortisol after 1-mg overnight dexamethasone suppression test was 39.5 g/dl. Pituitary MRI revealed an image suggestive of an intraparenchymal pituitary cyst. A thorax CT scan showed old fractures of some ribs bilaterally, without pulmonary nodules. Bilateral inferior petrosal sinus sampling (BIPSS) revealed a central-peripheral gradient >3. He underwent transsphenoidal surgery, and histological examination confirmed an adenoma with diffuse immunostaining for ACTH and focal for GH. The first day postoperative cortisol level was 5.0 μg/dl. He was started on replacement therapy with hydrocortisone and was oriented to Orthopedics appointment for surgical treatment of vertebral fracture.
Discussion
The investigation of osteoporotic fractures and secondary causes of hypertension was essential for the diagnosis of CS, considering that clinical features of CS were scarce. Hypercortisolism etiology was established after ACTH determination and BIPSS, which allowed adequate therapeutic guidance. Several studies have demonstrated that resolution of hypercortisolism is associated with increased bone mass, although the recovery is often slow and incomplete.

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P244

Cerebrospinal meningitis in 30-year-old man as first manifestation of pituitary macroadenoma

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Introduction
The most frequent clinical manifestations of pituitary macroadenoma include headache, vision disturbances and cranial nerve paralysis.
Case study
Thirty-year-old man was admitted to intensive care unit at regional hospital. On admission, he was unconscious, with convulsions, spasms and 3-day long history of headache as well as body temperature up to 41.5 °C. The patient was transferred to the Department of Infectious Diseases, with the suspicion of neuroinfection. MRI findings suggested the presence of pituitary abscess and extensive pathological lesion with the size of 27×28×38 mm with intensive marginal contrast enhancement, located in the sellar-suprasellar region. The lesion protruded into the sphenoid sinus through lowered Turkish saddle. Fluid content was also found in the sphenoid sinus. After 10 days of antibiotic therapy, the patient was transferred to neurosurgery ward for surgical treatment. Partial evacuation of pathological lesion was performed during right frontotemporal craniotomy. Patient's general condition following the surgery was moderately severe; the patient was conscious and able to react to simple instructions, he had left paresis affecting particularly left lower limb and he also experienced speech disturbances. Signs of hypopituitarism in all hormone axes were found and the patient was referred to the Department of Endocrinology at Pomeranian Medical University for further treatment. Follow-up MRI showed persistent pathologic mass in the sellar-suprasellar region, which penetrated sphenoid sinus through destroyed saddle floor. Once hormone deficiency has been corrected and the patient completed several weeks of antibiotic therapy, he was transferred to the Department of Neurosurgery at Pomeranian Medical University for further surgical treatment. Transspehnoidal resection of sellar-suprasellar tumour and sphenoid sinuplasty were performed. Histopathologic findings confirmed the diagnosis of pituitary adenoma. The patient was referred to rehabilitation unit. One year later, check-up MRI showed deepened Turkish saddle filled with a mass corresponding to post-operative material. There was no evidence of recurrent proliferation process.

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P245

Ectopic acth dependent Cushing syndrome diagnosed with octreotide scan

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Ectopic secretion of corticotropin(ACTH) by nonpituitary tumors accounts for 10–15% of ACTH dependent Cushing's syndrome (CS). Generally it is difficult to localize the ACTH secreting tumor by conventional imaging methods because these tumors are often small in size. Here we present a case of ectopic ACTH syndrome diagnosed with octreotide scan.
Case
A 26-year-old male patient presented with moon face, purplish striae, supraclavicular fat pads and proximal muscle weakness. In laboratory examination; early morning cortisol: 33 μg/dl (6.2–19.4), late evening serum cortisol: 30 μg/dl (2.3–11.9), serum potassium: 2.3 mmol/l (3.5–5.1), and urinary cortisol excretion: 258 μg/day (0–60). There was no cortisol supression with low dose or high dose dexamethasone. As plasma ACTH concentration was 92 pg/ml (0–60) the patient was diagnosed with ACTH-dependent CS. Pituitary MRI revealed no mass. Inferior petrosal sinus sampling(IPSS) was negative with no increase in ACTH after CRH administration. On thorax CT there was a 14 mm nodule in the middle lobe of the right lung. There was no pathology on 18-FDG-PET scan. But 111-In-Octreotide scan depicted the neuroendocrine tumor located in the right lung. In the lobectomy specimen the pathology of the mass was compatible with classic type carsinoid.

Conclusion
The last step of approaching to a patient with ACTH dependent CS is to detect the source of ACTH secretion. As most of the ACTH secreting tumors arise from thorax it is important to start evaluation from this anatomic localisation. CT or MRI is the first imaging modality of choice. The sensitivity of CT to detect a small tumor such this is 53% whereas it is 37% in MRI. The sensitivity of octreotide scan in detecting ACTH secreting tumor is approximately 30–53%. It is the last but not least choice of non invasive procedure on the way going to surgery as in our case.

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P246

Thalassemia minor associated with multiple pituitary hormone deficiency

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Regular red blood cell transfusions for thalassemia major leads to iron overload in endocrine glands, inducing deficient function of the pituitary, thyroid, parathyroid etc. In thalassemia intermedia these endocrine disturbances may appear due to increased intestinal iron absorption and occasional transfusions, but are less frequent and milder. We have no data about their presence in thalassemia minor. We present the case of a young woman known with mild hypochromic anemia since the age of 16. Her mother and brother have chronic anemia, too. She received iron therapy orally a few times, and parenterally 2–3 times, without the cause of anemic syndrome being clarified. She gave birth to a single child, and menstruations ceased at 29-years of age, after a prolonged psychological stress. Endocrine evaluation was performed after two years of secondary amenorrhoea, the 31-years-old patient presenting also memory loss, constipation, cold intolerance, flushes of heat, asthenia, intermittent headache. Physical examination showed short stature, pale skin, rare sexual hair, RR: 80/60 mmHg, HR: 60 bpm. Laboratory detected low or subnormal level of pituitary and corresponding peripheral hormones (TSH: 0.48 mIU/ml, normal: 0.38–4.31; fT_4: 0.35 ng/dl, *n*: 0.82–1.63; LH: 6.6 mIU/ml, *n*: 4.5–11; FSH:1.8 mIU/ml, *n*: 1.7–13.3, estradiol: 0.8 pg/ml, *n*: 40.7–220.4, 8 AM cortisol at baseline: 7.25 μg/dl, *n*: 6.4–21, cortisol after long Synacthen-test: 39.59 μg/dl, PRL: 10.9 ng/ml, *n*: 4.1–28.9), anemia (Hb: 9.9 g/dl, HCT: 31.3%, MCV: 65.2fL, MCH: 20.6 pg, Hgb electrophoresis: HbA1: 93.5%, HbF: 0.9%, HbA2: 5.6%). The pituitary CT-scan was normal. L-thyroxine (50mcg/day) and sexual hormone replacement therapy (2 mg/day estradiol for 21 days, and 2×100 mg/day progesterone for 10 days) were started, followed by artificial menstrual cycles, without aggravation of the anemic syndrome. Mild hyperprolactinemia (PRL: 56.5 ng/ml) with galactorrhea appeared, and the dose of estradiol was reduced to 1.5 mg/day, the clinical and laboratory status being under control now. We discuss the possible explanations of the association between two rare diseases: thalassemia minor and pituitary failure of apparently non-organic etiology.

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P247

Occurrence of De Quervain's thyroiditis after resolution of hypercortisolism following SOM230 treatment for Cushing's disease and surgery for an adrenocortical adenoma: report of two cases

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Introduction
Increased prevalence of thyroid autoimmunity has been described in few cases after successful treatment for Cushing's syndrome. In De Quervain's thyroiditis (DQT) autoimmunity does not seem to play a primary pathogenetic role.
We describe two cases of DQT coinciding with the resolution of hypercortisolism, after successful treatment of Cushing's syndrome/disease.

Case 1
A 41-year-old female with Cushing's disease who refused neurosurgery was started on SOM230. On the third month of successful treatment (UFC = 4 μg/24 h), she experienced a febrile state accompanied by malaise and severe neck pain. Clinically, thyroid was tender at palpation and tachycardia was noted. Laboratory tests revealed thyrotoxicosis (TSH = 0.065 mU/l, FT_4 = 2 ng/dl), and elevated ESR (110 mm/h). Thyroid uptake was absent on a technetium thyroid scan and thyroid ultrasonography was indicative of DQT. SOM 230 was temporarily discontinued, but due to persistence of fever and pain, despite non-steroidal anti-inflammatory medication, glucocorticoid treatment with methylprednisolone was initiated, resulting to a dramatic clinical improvement.
Case 2
A 50-year-old female patient with Cushing's syndrome due to a cortisol secreting adenoma had undergone unilateral adrenalectomy and been placed on glucocorticoid substitution due to HPA axis suppression. Five months post-operatively, at an attempt to taper hydrocortisone dose, she developed left sided neck pain, irradiating to jaw and ear, accompanied by low grade fever and malaise. Laboratory tests showed thyrotoxicosis, high ESR and negative thyroid auto-antibodies. Thyroid ultrasonography and scintigraphy confirmed the diagnosis of DQT. She was treated with prednisolone resulting in immediate symptomatic relief.
Conclusions
The incidence of DQT upon successful therapy of hypercortisolism, either medical or surgical has not been previously described. The exact pathogenetic mechanism could only be speculated. Perhaps the relative or absolute glucocorticoid deficiency, after effective treatment of hypercortisolism, alters immunologic responses and renders patients more vulnerable to thyrolytic processes.

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P248

Nephrogenic systemic fibrosis: potential aetiology of pituitary stalk thickening post- commencement of dialysis: case report

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Non-neoplastic pituitary stalk thickening is rare in patients without infiltrative disorders and diabetes insipidus. We present a non-diabetic patient with end stage renal failure with hyperprolactinaemia and pituitary stalk thickening.
A 53-year-old Nigerian gentleman presented with reduced libido, erectile dysfunction and painful gynaecomastia. He denied galactorrhea, headaches or visual disturbances. He previously received spironolactone for resistant hypertension and soon commenced dialysis for his end-stage renal failure investigations showed low testosterone level of 6.3 and elevated prolactin levels of 2683 which was attributed to hypogonadism secondary to ESRF. Serum oestradiol was 48, FSH 9.2, and LH 0.9. MRI pituitary in 2009 showed an expansion in the pituitary stalk measuring 5 mm with minimal mixed signal.
He was commenced on Bromocriptine which normalised his prolactin. He improved symptomatically, though his gynaecomastia persisted. In 2012, the patient stopped Bromocriptine due to nausea. He commenced of cabergoline and testim gel. A repeat MRI in 2012 showed no interval change despite the commencement of dopamine agonists.
Subsequent review of pre-dialysis MR renal imaging showed a small left kidney with an absence of renal artery stenosis. Investigations to determine the aetiology of his hypertension showed an elevated standing aldosterone/renin ratio of 2181 with normal adrenals on CT imaging; rendering his hypertension as essential.
Recent literature notes that patients in dialysis-dependent kidney failure are at significant risk for a recently described scleroderma-like disorder called nephrogenic systemic fibrosis. Nephrogenic systemic fibrosis (NSF) is associated with dermopathy and multi-organ dysfunction. No prior reports note pituitary involvement. Gadolinium-based contrast agents have been implicated in the development of nephrogenic systemic fibrosis. Our patient had an elevated prolactin before receiving gadolinium for his MR Renal Angiogram prior to commencing dialysis. We hypothesise his pituitary stalk thickening, which did not change post-bromocriptine is secondary to NSF.

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P249

Extraadrenal paraganglioma: case report

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The presence of pheochromocytoma/paraganglioma can be characterized by typical clinical signs, due to hemodynamic and metabolic activity of circulating catecholamines or less a consequence of other amines or neuropeptides produced simultaneously. But the clinical picture can be also very different and can play a clinical picture of other diseases.
The authors present a case report from a patient with retroperitoneal paraganglioma. The patient was admitted to our unit because of high blood pressure, hyperglycemia and weight loss. The reason for the CT was differential diagnosis of cachexy. CT found a solid body left paraaortal retroperitoneal tumor (size 8.5×4.5×5.5 cm), which was insistent on the aorta and renal artery and had necrotic disintegration. The patient subsequently passed the examination of adrenal scintigraphy with ^{123}I-MIBG, which described paraganglioma left paraaortal, radiopharmaceutical uptake was present in both adrenals and one lymphatic nodule in mediastine – the impossibility of exclusion metastatic malignant pheochromocytoma. We performed PET-scan, with the picture of present bearing signs of central necrosis in the retroperitoneum (left paraaortal) with only a low 18-FDG metabolism, and no other pathological changes in relation to the underlying disease on PET/CT image. To exclude MEN 2A syndrome we examined parameters of the thyroid gland (also with USG control), where we found the presence of cystic bearing (size 7.6×11.5 mm). Puncture aspiration biopsy of the node showed adenomatous node with cystic changes. The patient underwent genetic testing, which excluded RET mutation-protooncogen. The patient was subsequently operated at Urology Department, the tumor was extirpated and the histological examination showed that this was a retroperitoneal extra-adrenal paraganglioma (with angioinvasion, perineural spread, and extension into fat) with a recommendation to continue to monitor the patient. To assess the residual findings we performed PET scan, which described pathological heterogeneous tissue with increased metabolic activity in the retroperitoneum in place of the original tumor. The patient was without clear symptoms of the underlying disease activity in PET/CT image of 18-FDG. The laboratory results as well as the PET results, we consulted with professor Karel Pacak, from NIH (National Institute of Health), Bethesda, MD, USA, in order to exclude a possible recurrence. The patient was hospitalized in NIH and passed through this hospitalization complete biochemical and imaging studies. Scintigraphy with ^{123}I-MIBG showed a normal distribution of MIBG activity in the salivary glands, lungs, liver, gastrointestinal tract and urinary bladder, without evidence neuroendocrine tumor FDG/PET radiopharmaceutical recaptured only slightly in the surgical bed in left periaortic field. At the same time by the NIH genetics was the patient tested for familial paraganglioma (mutation succinatdehydrogenase subunits B and D), which has not been confirmed. Comprehensive examination of disease recurrence was ruled out for now.
Our case report will show that paranganglioma can be an 'actor' of clinical picture. In the diagnostic process is today also genetic examination very necessary, because of the knowledge of new gene mutations in the pathogenesis of endocrine diseases.

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P250

Newly discovered adrenal tumor and pituitary microadenoma in a patient known with classical congenital adrenal hyperplasia

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Introduction
Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder, most commonly caused by CYP21A2 mutations, and characterized by disturbed cortisol and androgen synthesis. Clinical manifestations of classical 21-hydroxylase deficiency are virilisation and salt-waisting syndrome in both sexes. Adrenal tumors may appear in untreated CAH patients, but very rarely following adequate long-term therapy.
Case report
The 17-years-old male patient was diagnosed with the salt-waisting form of CAH shortly after birth by biochemical (severe hyponatraemia 108 mEq/l and hyperkalaemia 7.8 mEq/l) and echographic findings (both adrenal glands almost 3 cm in diameter) after presenting two episodes of severe dehydration. He is under steroid replacement therapy since then. 21-Hydroxylase deficiency was confirmed by genetic testing in March 2011 (homozygote splice mutation in the 2nd intron). He has normal physical and mental development (in August 2012 weight 72.4 kg, height 172.2 cm, BMI 24.54 kg/m^2). Blood pressure, natrium (136 mEq/l) and potassium levels (4.4 mEq/l) were normal under hydrocortisone 40 mg/day. Repeated DHEA-S level is normal (2010: 63.4 mg/dl, *n*: 20–357; 2012: 90.9 mg/dl, *n*: 80–560), whereas 17-OH-progesterone levels remained high (2010: >19.20 ng/ml, *n*: 0.2–2.3; 2012: 41.53 ng/ml, *n*: 0.5–2.1). On the yearly follow-up cortisol levels were low (2010: 2.58 μg/dl, *n*: 6.2–19.4; 2012: 0.95 μg/dl) but ACTH levels got gradually higher (in 2010: 115.7 pg/ml, *n*: 7.2–63.3; 2011: 706 pg/ml; 2012: 2000 pg/ml). In 2012 abdominal CT showed a 21×15 mm oval-shaped nodule, with solid density (43 HU) on the left adrenal gland, pituitary MRI was suggestive for a 3 mm microadenoma, and cortisolemia was high (1.083 nmol/l, *n*: 171–536), besides high ACTH (452.2 pg/ml, *n*: 7.2–63.3) and 17-OH-progesterone (129 ng/ml, *n*: <1.39) level, that rose the suspicion of an ACTH-producing adenoma. The possible causes of these changes are discussed.
Conclusion
This case represents a very rare evolution of classical CAH that after 17 years of proper glucocorticoid therapy complicates with a left adrenal tumor and a pituitary microadenoma.

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P251

Malignant schwannoma in a patient with hypopituitarism, congenital hydrocephalus, atrial septal defect and agenesis of right kidney

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Introduction
Schwannomas are tumors derived from myelin sheat of nerves which can displace and compress nerves causing pain, weakness and numbness. Very rarely they become malignant.
Case report
Thirty-one years old patient was hospitalized at our Department to evaluate hypopituitarism. He was born at term by cesarean section, with hydrocephalus and did not start to breath spontaneously. A few months later, he was diagnosed with scoliosis thoracalis duplex and ostium secundum atrial septal defect. Hydrocephalus spontaneously resolved at the age of four and at the age of five he was diagnosed with hypopituitarism – GH deficiency, hypogonadotropic hypodonadism and hypothyroidism. Endorinological testing showed adequate cortisol response in ACTH test and insulin tolerance test. At the age of nine due to congenital spinal curves he underwent a surgery but significant spine gibus remained. At ten, he was diagnosed with Asthma bronchiale and congenital heart defect was surgically treated. From 11th to 16th year of age while on GH replacement therapy he grew from 92 to 154 cm. At the age of 12, a CT scan of the head was performed: Empty sella and a CT scan of the abdomen: agenesis of the right kidney. At the age of 27, due to a constant pain in his left shoulder and MRI was performed. It showed a tumefaction of 16×10×8.5 cm, located predominantly in the musculus infraspinatus and musculus teres minor and enlarged regional lymph nodes. The surgery was performed and pathohistological diagnosis was schwanoma malignum, low grade, with zones of myxoid transformation. Immunohistochemistry was performed in search for androgen receptors. The result was negative and his regular androgen therapy was continued. He has regular check ups at the Orthopedic Clinic with no signs of relapse. He takes the advised hormonal subsitution therapy and in general feels well.
Conclusion
Malignant schwannomas are very rare and are usually associated with neurofibromatosis type I. According to the available literature our patient is the first with the occurance of malignant schwanomma with hypopituitarism.

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P252

Non-Hodgkin's lymphoma presenting as anterior hypopituitarism

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Introduction
Panhypopituitarism is a known but rare presentation of lymphoproliferative disease. We describe a patient with newly diagnosed advanced diffuse large B-cell lymphoma who presented with anterior hypopituitarism and concomitant adrenal gland involvement.
Case report
A 72 year-old Chinese woman presented with a few weeks' duration of lethargy, weight loss and functional decline. A hormonal screen done at admission suggested central hypothyroidism, and an MRI of the brain performed showed pituitary stalk thickening without abnormalities in the size and morphology of the gland. Evaluation of the other hormonal axes revealed the presence of central hypocortisolism, secondary hypogonadism and low IGF1 levels. In addition, a computed tomography scan done as part of work-up for a right hilar mass seen on a screening chest radiograph revealed bilateral adrenal masses with surrounding enlarged para-aortic lymph nodes. Histology of the adrenals was diagnostic of a diffuse large B-cell lymphoma and a work-up for other causes was inconclusive. Our patient was not subjected to a pituitary biopsy as there was sufficient evidence of lymphomatous infilitration of the gland from the clinical findings. She was started on six cycles of R-CHOP chemotherapy along with thyroxine and glucocorticoid replacement. The patient had no features of diabetes insipidus before or after treatment. With the completion of chemotherapy, there has not been any functional recovery of the hormonal axes and our patient is currently still receiving hormonal replacement.
Conclusion
Panhypopituitarism is a rare but important aetiological consideration in the older patient presenting with functional decline. Our patient's presentation of isolated anterior hypopituitarism was unusual, as secondary lymphomatous involvement has a tendency to affect the posterior lobe instead due to its direct blood supply from the systemic circulation, unlike in the anterior lobe.

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P253

A case of ACTH producing oat cell carcinoma cause of ectopic Cushing's syndrome and life threatening hypokalaemia

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Introduction
Ectopic Cushing's syndrome caused by ectopic ACTH secretion are under-diagnosed.
Case report
A 50 years old male patient is hospitalized for severe hypokalaemia and diabetes. Last 6 months he had a history of chest pain, prolonged cough, general weakness, difficulties climbing stairs, confusion, loss of consciousness. Previously, he was hospitalized in psychiatric hospital for psychotic alterations, in cardiology for high blood pressure and cardiomyopathy (Ef 40%). Type 2 diabetes is diagnosed before 3 months; treated with basal insulin. Initial investigations revealed Na 144 mmol/l (135–145), K^+ 1.9 mmol/l (3.5–5.2), Urea 9 mmol/l, Cr(s) 47 mmol/l, Glu(s) 9 mmol/l. DXA confirmed osteoporosis (T-score > -2.5). Basal cortisol 1300 nmol/l, Daily rhythm of cortisol during 24 h was $>$ 1750 nmol/l. ACTH 641.2 pg/ml (7.2–63.6). On chest x ray it was suspicious tumoral mass, confirmed with chest CT. Tumor mass was present on upper mediastinum to the left until hilus, enlarged mediastinal lymph nodes, infiltration on the left lobe and pleural effusion in the same part. Gas analysis: partialy manifested respiratory insufficiency. Endoscopic bronchoscopy with ultrasound (EBUS) was performed for cyto/immunocytochemical analysis for ACTH. Citologycaly confirmed IV classification group of malignancy – oat cell carcinoma and immunocytochemy for ACTH was positive in 5–10% of neoplastic cells and positive for chromogranin in 30–40% and NSE in 50–60% of tumoral population. Patient was referred to Clinic of Pulmology and Institute of Oncology.
Conclusion
Diagnosis of ectopic Cushing's syndrome explain other co-morbidities such as hypokalaemia, diabetes, osteoporosis, psychosis. Appropriate diagnostic procedures without additional tests (dexamethasone suppression test, MRI), accelerates the diagnosis of this life-threatening patient.

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P254

Recurrent pituitary tumor: the importance of a functional classification at diagnosis

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Introduction
Pituitary tumors can be classified according to their endocrine function, starting from the clinical phenotype to establish a diagnosis. The histological analysis can confirm the clinical suspicion. A thorough classification is essential in the therapeutic approach with an important influence in the disease-free survival.
Case report
We report a case of a 16-year-old male, with loss of visual acuity and headache, attended by an Ophthalmologist in May/1993 who detected papilloedema and suggested a CT that showed a sellar tumor with 4×2.8 cm. He was subjected to surgery in December/1993. Histological diagnosis was 'non-functioning pituitary tumor'.
The post-surgical evaluation showed delayed puberty (TannerP1G1) and bitemporal hemianopsy. Laboratory findings: deficit in the thyroid, gonadal, adrenal axis and diabetes insipidus, with normal GH, IGF1 and prolactin. MRI showed 'intra and infrasellar tumor residue'. He was started on substitution therapy.
Beginning in late 1995, prolactin progressively rose to 152 ng/ml ($n < 18$ ng/ml), whereby in 1996 the patient was started on bromocriptine 2.5 mg, b.i.d. In 2000 was detected GH = 7.0 mUI/l ($n < 4$ mUI/l) with IGF1 = 377 ng/ml ($n = 182$–780 ng/ml), a 15 cm stature growth, without acromegaly symptoms or dysmorphism. Revision of the histological analysis: 'Prolactin secreting pituitary tumor'.
Persistently high random GH = 5.09 ng/ml ($n < 4$ ng/ml) and the elevation of IGF1 = 502 ng/ml ($n = 117$–319 ng/ml), motivated the initiation of somatostatin analogues in 2005. There was a favorable laboratorial response: GH = 3.41 ng/ml ($n < 4$ ng/ml), IGF1 = 292 ng/ml ($n = 117$–329 ng/ml) with reduction of the tumor residue dimensions.
At the present time, the patient has 34-years-old, a reasonable QoL under substitutive therapy, bromocriptine 10 mg, q.d., lanreotide 120 mg, every 6 weeks. The hormonal secretion is controlled: GH = 1.3 μg/l ($n < 1.0$ μg/l), IGF1 = 334 ng/ml ($n = 115$–307 ng/ml), prolactin = 10 ng/ml ($n < 18$ ng/ml), but the latest MRI performed in 2012 showed a tumoral residue of similar dimensions. He was proposed for radiation therapy.
Conclusion
This case illustrates the difficulties in the characterization of some pituitary tumors (either clinical, functional and pathohistological classification), specially in younger patients. The appreciation of the hormone levels must be integrated, considering the pubertal staging and not only chronological age.

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P255

Congenital trans-sphenoidal meningocele: an uncommon cause of pituitary insufficiency

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Introduction
Congenital trans-sphenoidal meningocele (TM) is a rare clinical entity. It can be traumatic, congenital or from tumour origin. It results from a defect in the sphenoid bone ontogenesis. In its classical form, the TM is revealed in adults, frequently in women in the fourth or fifth decade by rhinorrhea or cerebrospinal fluid leak through a bony defect in the sphenoid floor. Our aim is to report a case of large TM without rhinorrhea, and as a part of midline abnormalities.
Case report
An adolescent girl aged 19, was sent to our department for diabetes insipidus. Her medical history showed that she was treated since the age of four for growth retardation related to an isolated GH deficiency, but without any cerebral exploration. At 18 she complained from polyuria and polydipsia. Clinical and

paraclinical examinations argued for diabetes insipidus with well proved GH deficiency. Other pituitary functions were preserved. Cerebral MRI described a large dehiscence of the sellar floor with passage of cerebrospinal fluid through the sphenoidal sinus and persistence of meningeal peripheral wall defining a transsphenoidal meningocele which appears to be a part of the midline abnormalities as it is associated to cleft lip and hypertelorism.
Unlike other cases reported in the literature, our patient does not have rhinorrhea which represents a high risk for meningitis which is a life threatening condition for patients with this congenital malformation.
Conclusion
Congenital transsphenoidal meningocele is a rare condition, but should be kept in mind as a part of midline abnormalities and as a cause of pituitary insufficiency, cerebrospinal fluid leak and meningitis.

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P256

Isolated ACTH deficiency with Brugada syndrome: a combination increasing the risk of fatal arrhythmia

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Introduction
Brugade syndrome (BS) is a cardiac disorder characterized by typical electrocardiograph (ECG) alterations and is known to have a high risk of sudden cardiac death (SCD). Isolated ACTH deficiency (IAD) is often associated with fetal arrhythmia. Previous reports have suggested that certain endocrine diseases might cause Brugada-like ECG forms. We present a case of IAD with BS who died suddenly.
Case presentation
Forty-one year-old man was referred to our department because of severe hypoglycemia in July 2011. An arrhythmia was pointed out at his annual medical checkup in 2010 and he was diagnosed as having BS by cardiologists. The catheter ablation was done twice. After his second ablation, he lost consciousness due to severe hypoglycemia and was referred to our department. The endocrinological investigation revealed severe disturbance in ACTH secretion and mild disturbance in GH secretion. Slight elevations of thyrotropin (TSH) were observed but anti-thyroid antibodies were negative. Both TSH and GH secretion were normalized after the replacement with hydrocortisone and so he was diagnosed as IAD. Five months after the beginning of the replacement therapy, he died suddenly.
Discussion
BS is based on genetic defect concerning cardiac ion channels. On the other hand, several reports indicate that changes of thyroid hormone and cortisol and/or hyponatremia caused by adrenal insufficiency could induce Brugada-like ECG forms through the functional disorders in cardiac ion channels. In addition, abnormalities of ion channel function have been found in some patients with Schmidt syndrome. These reports suggest that IAD could influence in cardiac ion chancel function in this case, though the detail of functional connection between these hormone and myocardial ion channels remains unclear in humans.
Conclusion
This case shows us possibility that IAD can increase risk of fatal arrhythmia through its actions to cardiac ion channels.

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P257

A case of primary adrenal failure diagnosed in *postpartum* period

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Introduction
The prevalence of primary adrenal insufficiency (Addison disease; AD) in pregnancy is unknown. Female patients with adrenal insufficiency are usually infertile and once get pregnant are at increased risk for preterm deliveries, Cesarean section and babies with low birth weights. Recognition of AD during pregnancy may be difficult as many of the clinical complaints like weakness, lightheadness, syncope, nausea, vomiting, hyponatremia, and increased pigmentation can also be found in normal pregnancies. Herein, we present a woman with AD recognized at *postpartum* period.
Case report
A 32-year-old woman admitted with the complaint of hyperpigmentation throughout her body. She has given birth a healthy -2670 gr boy- at 36 weeks of gestation by vaginal delivery about 10 weeks ago before admission. The hyperpigmentation had started at the second trimester. She had no nausea and vomiting during gestation except the first trimester. At physical examination, there was generalized hyperpigmentation of the skin and knuckles, toes, elbows, knees, palmar creases, nail beds, nipples, buccal mucosa and gums. She had a blood pressure of 105/70 mmHg and serum sodium, potassium, chloride and glucose were found to be at normal ranges. Morning serum cortisol was 5.73 μg/dl with a plasma ACTH of 1250 pg/ml. She did not respond to insulin-hypoglycemia test. Bilateral adrenal enlargement was present on abdominal CT examination. A diagnosis of AD was made and she was started on hydrocortisone therapy. It has been proposed that fetal cortisol production may be protective for the mother from severe adrenal insufficiency until the *postpartum* period. This may explain why our patient did not suffer an adrenal crisis during pregnancy. Exacerbation of autoimmunity in the *postpartum* period may be another explanation for our patient.
Conclusion
Patients with subclinical adrenal insufficiency at early stages of the AD may present with a rather normal blood pressure and laboratory testing; so hyperpigmentation may be the only symptom in the patient.

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P258

TSH secreting pituitary adenoma: a case report

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Introduction
Thyrotropin secreting pituitary adenomas are rare constituting $<2\%$ of pituitary adenomas. Thirty percent of these tumors may be plurihormonal. Most common cosecreted hormone is GH and the least one is PRL. We report here a case of plurihormonal pituitary adenoma symptomatic for TSH secretion.
Case report
A 35-year-old female admitted to hospital because of fatigue, heat intolerance, headache, galactorrhea and menstrual irregularity. Her laboratory analysis showed hyperprolactinemia (PRL: 74 ng/ml), and high fT_3 level with inappropriately normal TSH (TSH: 3.14 μIU/ml (*n*: 0.27–4.2 μIU/ml) fT_3: 4.67 pg/ml (*n*: 1.96–4.36), fT_4: 1.24 ng/ml (*n*: 0.72–1.56 ng/dl). GH level was also high but IGF1 levels were all normal and she did not have any signs or symptoms of acromegaly. Pituitary MRI showed 18×15 mm macroadenoma extending to suprasellar region. She was followed-up with cabergolin for a year. Then she had transsphenoidal hypophysectomy. The immunohistochemical staining showed that tumor cells were strongly reactive to GH, PRL and TSH; Ki67 index was 2%. Four months after operation, she got pregnant. There was no biochemical abnormality during pregnancy. Her pituitary MRI didn't show any residual image. But at *postpartum* 2 months, high fT_3 and fT_4 levels were revealed. PRL was 60 ng/dl (analysis was done 4 h after breastfeeding), fT_3: 4.39 pg/ml, fT_4: 1.66 ng/dl, TSH: 2.33 μIU/ml, GH: 2.09 ng/ml, IGF1: 205 ng/ml (*n*: 109–284), cortisol was suppressed after dexamethasone suppression test (1.57 μg/dl). Physical examination revealed only tachycardia. Thyroid autoantibodies were all negative. Thyroid USG was normal other than a 5.3 mm heterogenous, isoechoic nodule. SHBG, glycoprotein alpha subunit to TSH ratio were normal. But TSH levels failed to increase after TRH stimulation test. A pituitary adenoma $16\times12\times12$ mm extending to optic chiasm, invading bilateral cavernous sinuses was found on MRI. Visuel field was normal. Octreotide LAR 20 mg/month and propranolol 40 mg/day were started and reoperation has been planned.
Conclusion
This case emphasizes that all pituitary adenomas should be screened for secretion of all hormones and immunohistochemical staining of all pituitary hormones is mandatory for correct classification.

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P259

Gastrointestinal bleeding associated with Dabigatran in a patient with panhypopituitarism

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Introduction
Dabigatran is a direct thrombin inhibitor which is used for reducing the risk of stroke or systemic embolism in patients with atrial fibrillation. Although INR monitoring is not required in the course of treatment, it may be complicated with spontaneous systemic bleeding. Sheehan's syndrome is characterized with panhypopituitarism which is caused by necrosis of the pituitary gland. Recent studies have demonstrated that panhypopituitarism is associated with bleeding disorder.
In the current study, we report a case with panhypopituitarism who suffered from gastrointestinal bleeding while using dabigatran.
Case report
A 74-year-old female patient admitted to the emergency department with the complaint of weakness and melena for the last 3 days. The physical examination revealed hypotension and tachycardia. The patient has been treated with dabigatran for paroxysmal atrial fibrillation for the last 1 month. She had no history of previous bleeding and antiplatelet treatment.
The laboratory test results were consistent with severe anemia (Hb: 3.8 g/dl), elongation of prothrombin time (15.1 s) and INR (1.37). Upper gastrointestinal endoscopy revealed multiply erosions in antrum. The patient developed profound hyponatremia (Na: 119 mEq/l) and progressive lethargy on the third day of follow-up. The advanced examination showed adrenal insufficency and central hypothyroidism.
Discussion
The Sheean syndrome has a very large spectrum of clinical presentation ranging from non-specific symptoms to coma. It may also lead to bleeding tendency rarely. Clinicians should consider this rare complication especially in patients who are to be treated with dabigatran.

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P260

Therapeutic dilemmas in a young male patient with macroprolactinoma complicated by hypogonadism

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Introduction
Hypogonadism is known to persist after treatment of hyperprolactinemia, necessitating androgen therapy in young male patients. Aromatisation of testosterone to estradiol can result in tumor expansion. We report a patient with persistent hypogonadism post treatment of macroprolactinoma and discuss challenges involved in the management.
Case report
A 51-year-old gentleman was admitted for streptococcal meningitis. Brain imaging revealed a pituitary macroadenoma with invasion into the cavernous sinus and erosion into the sphenoid sinus resulting in persistent cerebrospinal fluid (CSF) rhinorrhea. Laboratory investigations: FSH <1 IU/l (RI 1–19), LH <1 IU/l (RI: 1–19), Total Testosterone <1 nmol/l (RI: 5–30), free T_4 14 pmol/l (RI: 8–21), TSH 0.51 mIU/l (RI: 0.34–5.6), Prolactin 12 867 mIU/l (RI: 77–274), Short synacthen test response (293à848à977 nmol/l). He underwent transphenoidal removal of the prolactinoma and post operatively was complicated by panhypopituitarism requiring thyroxine and hydrocortisone replacement. A weekly dose of 2.5 mg of Cabergoline was needed to normalise prolactin levels. He reported low mood, libido and erectile dysfunction post surgery. FSH, LH and testosterone remained undetectable. He was started on i.m. testosterone at 100 mg every 4 weeks with normalisation of testosterone levels to 26 nmol/l and resolution of hypogonadal symptoms. His prolactin levels, expectedly, increased to 343 mIU/l. There were no signs and symptoms of raised intracranial pressure or cavernous sinus syndrome.
Discussion
Hyperprolactinemia suppresses the pulsatile secretion of GnRH resulting in hypogonadism. Estrogen, from aromatisation of testosterone, stimulates prolactin secretion by stimulating prolactin gene transcription and disrupting the inhibitory influence of dopamine. We discuss benefits and problems of various management options should there be tumor expansion post initiation of testosterone. These include increasing the dose of cabergoline or the addition of an aromatase inhibitor. Regular prolactin measurements and imaging of the pituitary are needed.

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P261

Baraitser Winter's syndrome and GH deficiency

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Introduction
Baraitser–Winter syndrome (BWS) is a rare brain malformation leading to droopy eyelids and intellectual disabilities. This syndrome, first reported in 1988, is probably due to genetic abnormalities that are still not well defined, although eight gene abnormalities are already discovered and *de novo* missense changes in the cytoplasmic actin-encoding genes ACTB and ACTG1 have been recently discovered (Riviere Nature Genetic 2012). The syndrome combines iris coloboma, bilateral ptosis, hyper telorism, broad nasal bridge, prominent epicanthic folds, brain malformations, and growth and mental retardation. To our best knowledge only 20 cases have been reported so far, among them five from Arab origin. Our aim is to describe the sixth Arab child with a phenotype that looks like Baraitser–Winter's syndrome.
Case report
A girl aged 7 years old of consanguineous parents was referred for short stature and mental retardation. Clinical examination showed dwarfism and a delay in mental development. Other clinical features included: strabism, epicanthic folds, broad nasal bridge, and brain anomalies such as lissencephaly, bilateral hygroma and cerebral atrophy. Hormonal exploration showed partial GH deficiency without other endocrine disorders.
Conclusion
Our case looks exactly like SWS. But, apart from facial and cerebral abnormalities, there is a partial GH deficiency which can explain the harmonious short stature. This case seems worth to be reported as it adds another abnormality to this very rare syndrome.

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P262

Beer potomania masquerading as adrenal insufficiency

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A 71-year-old male ex-publican presented to the Medical Emergency Unit suffering from lethargy, weight loss, dizziness on standing and dyspnoea on exertion. He had a past medical history of hypertension, ischaemic heart disease and alcoholic liver disease and he admitted to drinking 100 units of beer per week. His anthihypertensive medications included lisinopril and hydrochlorthiazide. On examination BMI was 35 kg/m^2, blood pressure 85/65 mmHg and there was no buccal or palmar hyperpigmentation. Admission venous blood tests revealed glucose 2.8 mmol/l and sodium 113 mmol/l. This led to a working diagnosis of Addison's disease. An MRI head, short synacthen test, ACTH and other baseline pituitary function tests were organised. MRI head revealed a structurally normal pituitary gland. The short synacthen test showed a low baseline cortisol of 109 nmol/l which rose to 510 nmol/l after 30 min. ACTH was 14 ng/l. All other tests were normal. Lisinopril and hydrochlorothiazide were stopped and he was commenced on i.v. hydrocortisone. This led to resolution of his hypotension and hyponatraemia. He was given a diagnosis of possible ACTH deficiency and discharged on replacement dose hydrocortisone to be followed up in the endocrine clinic. A repeat short synacthen test 3 months later showed a normal baseline cortisol of 513 nmol/l rising to 588 nmol/l after 30 min. Hydrocortisone

therapy was stopped and hyponatraemia improved with changes to his antihypertensives and a reduced alcohol intake. The cause of the patient's initial presentation was a combination of beer potomania and thiazide diuretic use. Beer potomania is an under-recognised condition characterised by hyponatraemia secondary to water intoxication. Recognition of this condition and careful fluid administration are required in the initial phase to avoid serious complications such as central pontine myelinolysis. Our case demonstrates that other causes of euvolaemic hypotonic hyponatraemia may obscure the actual diagnosis of beer potomania resulting in incorrect management.

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P263

Isolated ACTH deficiency associated with Hashimoto's disease

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Introduction
Isolated ACTH deficiency (IAD) is a rare disease which is characterized by secondary adrenal insufficiency with low cortisol production and normal secretion of pituitary hormones except ACTH. Isolated ACTH deficiency has rare association with Hashimoto's disease which is characterized by autoimmune origin. This suggests the possibility of common autoimmune process affecting both the pituitary and the thyroid gland. We report a case of IAD with Hashimoto's disease in a patient who presented with anorexia, nausea, vomiting and weight loss.
Case report
A 84 year-old man presented with anorexia, nausea, vomiting and weight loss for the last 3 months. His endoscopy revealed a Mallory-Weiss tear in esophagus. Sclerotherapy was performed. The laboratory test established hypothyroidism with plasma levels of free T_3 of 1.89 pg/ml (1.71–3.71), free T_4 of 0.47 ng/ml (0.7–1.48) and high TSH of 19.2 μIU/ml (0.35–1.94). The autoimmune antibodies were positive and the patient's level of cortisol was found 1.22 μg/dl. ACTH levels were determined as 3.4 pg/ml. The patient was diagnosed with secondary adrenocortical insufficiency. We examined the other pituitary hormones such as FSH, LH, prolactin and GH which were in normal limits. DHEA-S level was found 18.7 μg/dl (E: 80–560). The magnetic resonance of pituitary imaging was normal. Hyrocortisone and L-thyroxine supplemention improved his symptoms.
Conclusion
Isolated ACTH deficiency is a rare cause of adrenal insufficiency which can be associated with Hashimoto's thyroiditis that may present with severe gastrointestinal symptoms such excessive vomitting.

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P264

Cases with adrenocortical carcinoma

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Introduction
Adrenocortical carcinoma (ACC) is a rare cancer (estimated incidence, 0.7 to 2.0 cases per 1 million population per year) with a poor prognosis. Even after seemingly complete surgical resection, most patients develop recurrence within 5 years. The 5-year survival rate is $<15\%$ among patients with metastatic disease.
Methods
We present the results of six patients with adrenocortical carcinoma followed in our clinic.
Results
The mean age was 54 years and female/male ratio was 2/4. Tumor size ranges from 6 to 14 cm. At baseline, only one patient had high dehydroxyepiandrosterone-sulphate levels (Patient 1). Tumor was non-functional in 5 patients. One patient presented with Cushing's syndrome and hypercortisolism was documented in her laboratory evaluations (Patient 4). All of them were treated with surgery. In one patient, tumor was bilateral and in others were on left adrenal. The patient with bilateral tumors had lung metastasis at diagnosis (Patient 3). Another patient developed lung metastasis 2 years after the operation (Patient 1). Patient 4 had lung metastasis and Patient 6 had lung, liver and lymph node metastasis. One patient was lost-to follow-up after the operation (Patient 5). Three patients died 5 months after the operation. Three out of six patients received mitotane after the operation. Two patients could not receive mitotane because of elevated liver enzymes (Patients 4 and 6). Patient 1 developed lung metastasis 2 years after the diagnosis and is now receiving chemotherapy (cisplatin-etoposide) with mitotane. This patient is being followed in our clinic for 53 months.
Conclusion
Treatment options for advanced ACC are limited. Even after successful complete excision of the tumor, local or distant metastatic recurrences are frequent. However, some patients on regular follow-up and mitotane as adjuvant therapy may benefit from treatment as seen in Patient 1.

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P265

Differential diagnosis of an incidental pituitary lesion detected with PET-CT in a patient with a known history of metastatic maxillary sinus tumor

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Introduction
Metastatic pituitary tumors are seen rarely and it is hard to differentiate them from the benign lesions of the gland. We have reported a case, with a known maxillary sinus tumor, detected to have a pituitary lesion coincidentally on PET-CT.
Case
45 years old male patient with a known history of relapsed maxillary sinus tumor has been referred to our clinics because of the pituitary lesion detected to have increased FDG involvement on PET-CT examination. He was operated for the maxillary sinus tumor 35 years ago and had the second operation last year because of the recurrence. Histopathological examination was reported as well-differentiated squamous cell carcinoma with perineural and lymphovascular invasion. There was a tissue defect on the right maxillary region, nose and the right eyelid in physical examination. Pitiutary lesion was metabolically active on PET-CT and SUV_{max} value was 11.7. We have demanded a pitiutary MRI and detected a 5.4×4.3 mm sized nodular pitiutary lesion on the right side of the gland, which was isointense on T1A and T2A images and with late contrast concentration on dynamic sequences. In laboratory examination, anterior pituitary functions are in normal ranges. There wasn't any symptom or sign of diabetes insipidus. Tissue biopsy for the definitive diagnosis could not be performed because of the facial defect.
Conclusion
It is difficult to differentiate metastatic lesions from the benign lesions of the pituitary gland. Most of the metastatic lesions are asymptomatic although presences of diabetes insipidus or ophthalmoplegia are suspicious for metastasis. Also, there isn't any specific radiologic sign for metastatic lesions. In our case because of the lesion being metabolically active on PET-CT with a high SUV_{max} value, isointense appearance on MRI and a history of maxillary sinus malignancy made us suspect pituitary metastasis.

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P266

Secondary hypertension and hirsutism as a clinical manifestation of the tumor duplicity: case report

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Differential diagnostic search for the pathogenetic causes of hirsutism and the combination of hypertension can provide very interesting results for the clinicians. The case report demonstrates a patient suffering from two hormonally active tumors – adrenal adenoma with primary aldosteronism and primary Leydig cell ovarian tumor with hyperandrogenism. The task of the authors was easier due to the perimenopausal age of the proband. Selective venous sampling was very helpful in diagnosis of these endocrine active tumors. Both of them were resolved by a single laparoscopic surgery. The combination of the two described tumors is the really unique clinical finding. The solution using laparoscopy in a single procedure provided an elegant and efficient therapeutic approach.

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P267

Treatment dilemmas of Cushing disease: case report

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Introduction
Cushing's disease (CD) is a Grave disease that requires a multidisciplinary and individualized treatment approach.
Case report
We describe the case of a 31-years old female patient with Cushing disease diagnosed in 2007. She initially complained of weight fluctuations, amenorrheia, rounded face with plethora and acne, for 1 year duration. Analytically: 0800 h plasma cortisol of 14 μg/dl (5–25) and 2300 h 15 μg/dl, ACTH 18 and 20 pg/ml, respectively; UFC 420–510 μg/24 h (10–80). Cortisol after LD-DST 3.4 μg/dl and after 2 days LD-DST 13 μg/dl. CRH test: plasma cortisol increased 20% and ACTH 60%. MRI: 5 mm lesion in pituitary left lobe. She was submitted to transphenoidal surgery (TSS) in 2/2008. Pathology: corticotrophinoma. After surgery: 0800 h plasma cortisol 9.4 μg/dl and 2300 h 4.1 μg/dl, ACTH 17 pg/ml and 15 pg/dl; UFC 86 μ/24 h; 2 days LD-DST cortisol <0.1 μg/dl. No residue in MRI. Follow-up until 11/2009 revealed fluctuating clinical symptoms with periods of depression, acne and weight gain. She also had osteoporosis and renal calculi. UFC: 45, 135, 85, 166 and 210 μg/24 h. Plasma cortisol after 2 day LD-DST: 6.6 μg/dl. MRI: possible small lesion, not confirmed in MRI of 3/2010. Nevertheless, in 4/2010 she was submitted to a second TSS, without any corticotrophinoma found. Hormonal evaluation (5/2010): plasma cortisol after 2 days LD-DST of 4.0 μg/dl, UFC 146–275 μg/24 h, no pituitary insufficiencies. Inferior petrosal sinus sampling study wasn't conclusive. MRI (11/2011) wasn't suggestive of microadenoma. Since then and until 8/2012, worsening of clinical manifestations: menstrual irregularities, acne, depression, supraclavicular fullness, body bruising and osteoporosis. UFC 330 μg/24 h, 0800 h plasma cortisol 15 μg/dl and ACTH 26 pg/dl. Abdominal CT (7/2012): adrenal glands without alterations. As she maintains active disease bilateral adrenalectomy was proposed, to preserve fertility.
Conclusions
CD remains a challenge at diagnosis and causal treatment. Simultaneously is associated with severe morbidity (and mortality) related with the severity but also with the duration of the hypercortisolism. For young patients preservation of gonadal function is an important issue, leading to the sooner option for bilateral adrenalectomy.

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P268

Adrenocortical carcinoma: a case report

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Adrenocortical carcinoma (ACC) is a rare (incidence 1–2 per 1 million population) and heterogeneous malignancy with incompletely understood pathogenesis, unknown etiology and poor prognosis. ACC tends to grow and metastasize rapidly if untreated. The majority of cases are incidental diagnoses of advanced stage disease that metastasizes precociously due to its tendency to invade vascular structures. Among adults presenting with hormonal syndromes due to tumor excess secretion, Cushing's syndrome alone is most common, followed by mixed Cushing's and virilization (glucocorticoid and androgen overproduction). Conn syndrome (mineralocorticoid excess) occur in $<10\%$ of cases. Our patients shows all symptoms of Cushing and Conn syndrome.
Case report
Patient is a 37-year-old woman with oncologic genetic load. Patient presented with spontaneous fracture of the right femur and transferred to our clinic because of suspicion of ACC. Patient has one year progression of high blood pressure controlled with angiotensin-converting enzyme inhibitor. She complained also of diffuse postprandial abdominal pain with peaks of intensity accompanied with vomiting, aside from high blood pressure manifestations of muscle cramps, muscle weakness and headaches. Our patients is also presented with signs and symptoms of hormonal syndromes (Cushing syndrome, virilism and Conn syndrome).
Laboratory work-up including full blood count, blood chemistry, serum electrolytes, liver function tests, cortisol, aldosterone, normetanephrines, total metanephrines, and urine metanephrines. Ultrasound imaging revealed $90\times83\times88$ mm left heterogeneous adrenal mass that was confirmed with computer tomography and magnetic resonance. Histopathological result of adrenal mass reported adrenal carcinoma.. Local invasion and tumor extension into the inferior vena cava as well as lymph node and left kidney or other metastases (lung and liver, peritoneum, colon, femur, mandible) are found. Palliative cisplatin-based chemotherapy and radiotherapy are alternative to surgical debulking. Ketoconazole is used to control hypercortisolemic symptoms and the therapy with i.v. bisphosphonate (Pamidronat 60 mg) is also given to prevent further pathological fractures.

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P269

Fountain of steroid deep within: a case report of an ectopic ACTH-producing tumor

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Background
Cushing's syndrome is defined as a hormonal disorder resulting from elevated serum cortisol leading to specific physical and biochemical abnormalities that might be detrimental to life itself. Majority is caused by steroid intake while endogenous sources although representing only a minority of patients often lead to delay in diagnosis. Ectopic ACTH syndrome considered a rare entity are usually found as small cell lung cancer and carcinoid tumors. Rare types of ectopic tumours are thymomas, pancreatic tumors, and medullary thyroid carcinomas. Knowing their rare presentations and diagnostic clues might help physicians in the future to easily diagnose and manage endogenous Cushing's syndrome since most are completely reversible.
Case summary
A previously healthy 39 year-old Filipino female presented with rapid development of features compatible with Cushing's syndrome (truncal obesity, buffalo hump, acne formation, moon facies, plethora, muscle wasting, acanthosis nigricans) in a span of 2 months. She also presented with metabolic alkalosis and significant hypokalemia leading to muscle paralysis. Significant hyperpigmentation of peculiar body sites (knuckles and toes) were observed. With further work ups and repeated hospitalizations, patient exhibited insulin resistance (secondary diabetes), hypertension, dyslipidemia and thyroid function abnormalities. Patient eventually referred to our institution for acutely worsening behavioural changes. Biochemical tests revealed elevated serum cortisol ($12\times$), unsuppressed high dexamethasone suppression test and an elevated serum ACTH leading to a suspicion of a possible Ectopic-ACTH syndrome. Abdominal CT showed multiple liver masses. Patient eventually succumbed to nosocomial infection. On autopsy, an ectopic well differentiated neuroendocrine tumor was found at the pancreatic head with metastasis to the right hepatic lobe.
Conclusions
Knowledge of this disease and certain clinical clues and features (rapid clinical course, metabolic alkalosis with hypokalemia, severe hyperpigmentation, behavioural change) can guide clinicians to earlier diagnosis, rapid tumor location and immediate therapeutic initiation that can be life-saving.

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Clinical case reports - Thyroid / Others

P270

Low TSH plasma levels and high fracture risk in postmenopausal osteoporosis and osteopenia patients

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Introduction
Bone loss in thyroid dysfunction was first described by von Recklinghausen. Elevated bone turnover and decreased bone mass result when thyroid hormone levels are high and TSH levels suppressed. The changes in bone mass are impressive even in patients whit subclinical hypertiroidism, where thyroid hormones are within the normal range, but TSH is low or undetectable. Evidence show that endogenous and exogenous TSH suppression is associated with an increased fracture risk.
Methods
We studied 1487 postmenopausal women (average age 65.47 ± 9.94). TSH (0.3 to 4.2 μUI/ml) were correlated with the prevalence of vertebral fractures, classified by morphometric study. Postmenopausal patients showed different thyroid functional status. They received DXA evaluation and fulfilled WHO criteria for Osteoporosis.
Results
Osteopenia was diagnosed in 587 patients (39.5%), osteoporosis in 875 (58.8%). In osteopenic group 148 women (10%) showed vertebral fractures, while 645 osteoporotic patients (42.7%) had fractures. In addition, 'major' vertebral fractures (>25%) were present in 170 patients (11.4%) with subclinical hyperthyroidism (TSH <0.3 μUI/ml). This result was compared to group of 60 women (4%) that showed TSH>0.3 μUI/ml (total percentage significant ($P<0.05$)). There was a correlation between subclinical hyperthyroidism and BMD *t*-score values (correlation, Spearman's $\rho=0.18$ is $P<0.001$).
Conclusions
Our data suggest the relationship between TSH plasma levels and vertebral fractures in women with postmenopausal osteoporosis and osteopenia. Because of the strong correlation between low TSH levels and high fracture risk, which appears to be dissociable for long-term decrements in BMD, we suggest maintaing TSH levels during replacement therapy to above 1 mU/ml, unless there is a clinical rationale for TSH suppression as in thyroid cancer patients. In these patients, admittedly without clinical evidence of efficacy, we propose the empiric use of an oral biphosphonate to prevent the high turnover osteoporosis and associated fracture risk.

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P271

Malignancy rate of thyroid nodules, which defined as follicular lesion of undetermined significance and atypia of undetermined significance in thyroid cytopathology and relation with ultrasonographic features

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Introduction
Fine needle aspiration cytology (FNAC) has been widely accepted the most accurate, safe and cost-effective method for evaluation of the thyroid nodules. The most challenging category in the FNAC is atypia of undetermined significance (AUS) and follicular lesion of undetermined significance (FLUS). Bethesda System (BS) recommends repeat FNAC in that category due to their low risk of malignancy. In our study, we aimed to investigate the malignancy rate of thyroid nodules of AUS and FLUS and to evaluate the presence of biochemical, clinical and echographic features possibly predictive of malignancy related to AUS and FLUS.
Materials and methods
Data of 268 patients operated for AUS and FLUS cytology were screened retrospectively. Ultrasonographic features and thyroid function tests, thyroid antibodies, scintigraphy and histopathological results were evaluated.
Results
Two hundred and seventy six nodules of 268 patient's results are evaluated. Malignancy rates were 24.3% in the AUS group, 19.8% in the FLUS group and 22.8% in both group. In the evaluation of all nodules the predictive features of malignancy are hypoechogenicity and peripheral vascularization of the nodule. In the AUS group, the predictive feature of malignancy is only hypoechogenicity, and peripheral vascularization in the FLUS group.
Conclusion
We determined that the malignancy rates in these nodules are higher than the anticipated literature rate. This high ratio may be due to the fact that we studied only patients who underwent surgery. The ultrasonographic features alone may be insufficient to predict the malignancy, therefore all the clinical and ultrasonographic features must be considered in the evaluation of the thyroid nodules. In addition, we think that, the recommended management of repeat FNAC in these groups must be reconsidered with the clinical and ultrasonographic features.

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P272

Recurrent pregnancy induced hypercalcaemia resulting in multiple terminations of pregnancy

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Introduction
Pregnancy is characterized by increased intestinal calcium absorption, normal ionized or albumin-corrected calcium, high calcitriol, low PTH, gradually increasing PTHrP, and hypercalciuria. These differing hormonal changes can lead to nonclassic presentations of disorders of bone and mineral metabolism.
Case report
First-trimester 34-year-old multigravida patient presented with hyperemesis, abdominal pain, polyuria, polydipsia, leg cramps and constipation. Hypercalcaemia (4.13 mmol/l) with suppressed PTH was found while she was not taking any calcium. 25OH-VitD was low (40 nmol/l) and ACE level normal. 24 h urinary calcium and serum1, 25OH-VitD were elevated. PTHrP was not consistently elevated which may be related to the reliability of the assay itself. Imaging of breast, trunk revealed no evidence of maternal malignancy. BMD scan Hip *T* score was −1.7 and *Z* score −1.5 but Lumbar spine was normal. I.v. fluid therapy led to brief symptomatic improvement. Course of prednisolone failed to suppress calcium. Safety of bisphosphonates in pregnancy not established. Decision was made for medical termination of pregnancy (TOP) at 17 weeks from lack of symptomatic control of hypercalcaemia and the concern of its effect on the foetus. Calcium, PTHrP and 1,25OH-VitD levels normalised post termination. Foetal autopsy revealed no abnormalities apart from placental membrane calcification. The risks of gestational hypercalcaemia in future pregnancies discussed. She conceived again and developed uncontrolled hypercalcaemia with elevated 1,25OH-VitD and PTHrp requiring termination at 12 weeks with normalisation of calcium. Placental analysis failed to show abnormal PTHrP staining. Extensive counselling undertaken regarding future pregnancies. There was no documented hypercalcaemia in previous pregnancies with former partner. She conceived twice again with a resultant miscarriage at 8 weeks and TOP at 7 weeks.
Discussion
The usual causes of hypercalcaemia were excluded. This appears to be a pregnancy-related phenomenon, and postulated mechanisms are either excessive calcium gut absorption due to increased sensitivity to 1,25 Vitamin D or an occult source. Could this be related to aberrant prolactin receptors with resultant high PTH and altered gene transcription. There were limited studies in 2009 assessing the role of bisphosphonates or other drugs in pregnancy.

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P273

Familial Graves' disease: case report

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Introduction
Graves' disease is an autoimmune condition with an estimated prevalence of about 2%, 5–10 times more frequent in females. A multifactorial nature is

assumed, with genetic contribution accounting for up to 80% of the variability. Relevant genes includegeneral autoimmune risk *loci*, such as the HLA region, CTLA-4, PTPN22 and CD40, as well as thyroid-specific loci (thyroglobulin and TSH receptor genes). We report a particular case of Graves' disease with a strong familial aggregation and speculate on possible immune mechanisms.
Case report
A possible founding effect occurred in the maternal grandmother with Graves' disease, apparently in the sixth decade of life. Two of her three children, male and female, presented with Graves' disease in the fourth decade of life. The clinically unaffected female child has two sons, both affected with Graves' disease in the first two decades of life. In no case was there evidence of exophtalmus or pretibial mixedema. In every case, the initial treatment consisted of antithyroid drugs metimazol or propyltiouracil maintained for two years and was interrupted when TRAb titers became negative. Recurrences after the first episode occurring in every case required definitive treatment (radioiodine or surgery).
Discussion
This family is affected by Graves' disease in three generations with no gender predominance suggesting a dominant autossomic pattern. It also appears to be genetic anticipation by means of a lower age of onset and an increase in severity with more rapid recurrences in succeeding generations. Accordingly, the clinically unaffected child in the second generation may also develop the disease. The fact that there is no evidence of other immune-mediated disorders or other immune processes directed against common target organs in Graves' disease (exopthalmus or pretibial myxedema) points to a thyroid-specific antigen, most likely the TSH receptor. Genetic and molecular analysis of the TSH receptor is presently underway.

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Paraganglioma, germline nonsense mutation in succinate dehydrogenase B gene (R27X) and response to sunitinib

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A 36-years-old male with mild hypertension was diagnosed with pseudocystic tumor (67 mm) in pancreatic region and liver metastasis in April 2010. One month later, the extirpation of retroperitoneal mass and metastasis was performed in local medical centre. Pathohistological diagnosis was metastatic paraganglioma (Ki67, 3.5%). In June 2010, he was transferred to our Institution, and (131) I-metaiodobenzylguanidine (MIBG) scintigraphy was negative. However ((18) F) fluorodeoxyglucose positron emission tomographic (18-FDG-PET) CT scanning showed on area (SVII) of uptake within the liver. In March 2011, multiple focal liver lesions were detected by MSCT scan, which were negative on In-111 octreoscan and MIBG scintigraphy. Urinary adrenalin, noradrenalin and dopamine were normal, while chromogranin A level was elevated. Germline nonsense mutation was identified in exon 2 (R27X) of succinate dehydrogenase B (SDHB) gene. Patient's father and younger brother were carriers of the same mutation.
At the same time period, we tested patient's, 2-years younger brother who carried the same germ-line mutation. He suffered from hypertension since he was fourteen. Retroperitoneal mass (9.5 cm) was detected at the age of seventeen by echography. In January 2008, extraction of retroperitoneal tumor was performed in local medical centre, and during the surgical procedure the rise in blood pressure was recorded. Histopathology showed malignant paraganglioma. He came to our clinic in May 2010 without any symptoms. Abdominal MSCT revealed liver metastases. Although MIBG scintigraphy and In-111 octreoscan did not show uptake, an 18-FDG-PET/CT showed multiple areas of uptake in liver. Urinary adrenalin, noradrenalin and CgA were normal and urinary dopamine was slightly elevated.
Both brothers continued treatment with sunitinib and partial remission was registered after 3 months.
This nonsense germline mutation in SDHB gene (R27X) is associated with the similar phenotype and the similar response after sunitinib administration in both patients.

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Submandibular ectopic thyroid gland

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Introduction
Ectopic thyroid glands are generally a rare entity appearing mostly in the cervical midline (90% of the cases) and even more rarely in other anatomical sites (10%).
Clinical report
We present the case of an ectopic submandibular thyroid gland. A 31-year-old woman was referred to our department with a history of left submandibular swelling.
A scintigraphy was performed, which showed an accumulation of activity in the right submandibular region without any evidence of further thyroid tissue in the normal site.
Preoperative FNA revealed benign ectopic thyroid tissue.
Hormonal laboratory test showed normal thyroid function.
The patient was submitted to resection of the mass. Histological examination of the specimen confirmed the diagnosis.
A substitution treatment with thyroxin was initiated postoperatively, as this was the only functional thyroid tissue.
Conclusion
Physicians should be aware of the possibility that a submandibular swelling could be an ectopic thyroid gland. This entity poses specific diagnostic and therapeutic difficulties. Thorough preoperative diagnostics and resection of the ectopic tissue guarantee a good outcome. Physicians should also consider that this ectopic gland is the only functional thyroid tissue in 70% of cases.

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Barraquer-Simons syndrome

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The Barraquer-Simons syndrome is a rare form of partial symmetric lipodystrophy with involvement of the face and upper body, of unknown cause, characterized by loss of subcutaneous fat in the face and started stretching for the upper body.
Case report
Female patient, 26 years old, presenting morphologic change in facies, chest, upper limbs, lower limbs and abdomen since she was 10 years old. Last year, she has increased weight at 30%, with apparent muscle hypertrophy, *clitorimegaly*, facial hypertrichosis and decreased libido. Physical examination shows normal thyroid palpation, absence of umbilical hernia and bone abnormalities. Menarche at 13 years, with regular menstrual cycles. Deafness and epilepsy were excluded.
Commentary
The Barraquer-Simons syndrome was described in 1904, predominantly affects females (4:1), and the appearance of symptoms commonly happens at the end of the first or beginning of the second decade of life. Its origin is unclear, but associations have been made with genetic mutations, neuroendocrine disorders and autoimmune diseases. Most cases appear to be isolated in their families. Occasionally, functional abnormalities such as deafness, epilepsy, mental retardation, neuropathy, myopathy, and vascular disease may be associated with dermatomyositis. Diagnosis is mainly clinical and their distinctions with other syndromes such as Cockayne, SHORT, Berardinelli-Seip syndrome and bilateral Parry-Romberg. Currently, facial reconstruction techniques have been used to restore facial contours, and the disease management must include the monitoring and treatment of complications, because it is a phenocopy of other syndromes such as lipodystrophy which presents diabetes and hypertriglyceridemia. Barraquer-Simons syndrome is quite rare disease, mainly producing facial changes, to which are directed most of the treatments, however studies should be conducted to clarify the pathophysiology of the disease.

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Dural ectasia accompanying a case of multiple endocrine neoplasia type 2B

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Introduction
Manifestations of MEN2B include medullary carcinoma of thyroid (MCT), pheochromocytoma, and a number of somatic mutations like marfanoid habitus, mucosal neuromas, ganglioneuromatosis of the bowel. Dural ectasia results from enlargement of the spinal canal, was identified in 63–92% of patients with Marfan syndrome, however, it was not previously described in MEN2B. We detected dural ectasia in our patient with MEN2B.
Case report
A 28 year-old woman was admitted with abdominal discomfort. Bilateral adrena masses were detected by ultrasonography. She had been operated for nodular disease and took a diagnosis of MCT before 13 years. At that time, she had not have pheochromocytoma and her adrenal glands were detected as normal. At physical examination, multiple neuromas on the lips and tongue, marfanoid facies were detected. A decreased upper/lower body ratio was present. Laboratory examination revealed elevated levels of urinary catecholamine metabolites (Normetanephrine: 12 789 μg/day, Metanephrine: 9650 μg/day). Magnetic resonance imaging showed bilateral adrenal masses (40×18 mm left, 50× 25 mm right) compatible with pheochromocytoma. Dural ectasia was detected incidentally at sacro-iliac region. Calcitonin level was detected to be normal, her neck ultrasonography was negative for recurrence of MCT. After bilateral adrenalectomy, she was started on hydrocortison and her urinary catecholamine metabolites were detected as normal.
Conclusion
Patients with MEN2B have development abnormalities, a decreased upper/lower body ratio, skeletal deformations, joint laxity, Marfanoid habitus, and myelinated corneal nerves. Disturbances of colonic function are common, including chronic constipation and megacolon. Dural ectasia can be seen in anchylosing spondylitis, achondroplasia, Loeys-Dietz syndrome and in the vascular form of Ehlers-Danlos syndrome besides Marfan syndrome. This abnormality results from enlargement of the spinal canal owing to progressive ectasia of dura and neural foramina and to erosion of vertebral bone, it involves lumbosacral spine. To our knowledge, this is the first case of coexistence of MEN2B and dural ectasia.

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Coexistence of euthyroid ophthalmopathy and isolated ocular myasthenia gravis in a patient with vitiligo: a challenging diagnosis of APS-3C

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About 1% of the patients with Graves' disease develop myasthenia gravis, however, euthyroid Graves' ophtalmopathy has only rarely been associated with myasthenia gravis and especially with isolated ocular myasthenia gravis. A 63-year old, male patient with vitiligo presented double vision and right ptosis, shortly followed by left ptosis. On the ophthalmological examination there was bilateral ptosis and orbito-ocular ultrasonography yielded a thickening of the left lateral recti. Electromiographic and extensive biochemistry examinations were normal. Thyroid dysfunction was absent with normal TSH receptor antibodies but antithyroid peroxidase antibodies titers were 82.88 IU/ml (normal <34). Thyroid ultrasound examination showed a nodular goiter presenting diffuse decreased echogenicity. The ocular manifestations were interpreted as euthyroid-associated orbitopathy in the context of Hashimoto thyroiditis and the patient received a course of corticotherapy. Two months later he was admitted with bilateral exophtalmos and sclera and conjunctival injection and received combined glucocorticoid and radiotherapy. Two weeks after the corticotherapy was stopped, the patient experienced exacerbation of diplopia and bilateral palpebral ptosis. At a new neurologic assessment, repetitive nerve stimulation of the ulnar nerve recording from abductor digiti minimi of the right hand revealed a decremental response and serum acetylcholine receptor antibody was positive at 5 nmol/l (normal <0.4). Chest X-ray and chest computed tomography did not reveal any thymic hyperplasia or thymoma. TSH was slightly reduced at 0.158 μU/ml and anti-thyroglobulin antibody levels were 845 IU/l (normal ≤ 115). Pyridostigmine and intermittent glucocorticoid treatment was recommended. At 2-years follow-up visit, no signs of generalized myasthenia were detected. We report a case of euthyroid Graves ophthalmopathy associated with isolated ocular myasthenia gravis appearing in a patient with longstanding vitiligo, compatible with an autoimmune polyglandular syndrome-3C phenotype. The coexistence of these two entities, in the absence of overt thyroid dysfunction or generalized features of myasthenia gravis may cause diagnostic confusion.

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Giant cell granuloma as initial presentation of primary hyperparathyroidism: a case report

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Giant cell granuloma is a skeletal manifestation seen now rarely in hyperparathyroidism due to early recognition of the disease. Lesions usually occur in the areas of intense bone resorption. They can affect mandible, maxilla, clavicle, ribs and pelvic bones. Most of the patients who have primary hyperparathyroidism are asymptomatic and are discovered incidentally during laboratory examinations. Here, we represent a female patient who was referred to endocrinology clinics because of maxillary brown tumor detected by her dentist.
Case
Thirty-years-old female patient has admitted to dental clinics with the complaint of oral mass and accompanying symptoms as decreased apetite, weight loss and numbness on the jaw. Biopsy of the oral lesion was consistent with giant cell granuloma. Radiographic imaging has revealed multiple bone cysts on the mandible and maxillary bones. In laboratory exam, severe hypercalcemia and hypophosphatemia were detected. Her serum parathormon level and urinary calcium level were elevated. In ultrasonographic examination, we have detected two parathyroid adenomas on left side and MIBI scan was positive. In bone mineral densitometry, osteoporosis was detected at the lumbar vertebrates. She didnt have any renal stones in abdominal ultrasonography. We have searched for MEN1 syndrome. There was a microadenoma in pituitary MRI that was proven nonfunctional with hormone tests. Her upper gastro intestinal endoscopy was normal as the serum gastrin levels. She underwent surgery and all parathyroid glands and thymus were excised then 1/2 of a parathyroid gland was seeded on forearm. No complications occurred during or after surgery and she was discharged and referred to her dentist for curettage of the bone cysts.
Treatment of parathyroidism is surgery. However, treatment for bone lesions is controversial. They can regress after removal of the adenoma. In the persistent cases, surgical removal of the bone lesion after a short course of steroid therapy is advised.

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Fibrous variant of Hashimoto's thyroiditis: development of neck fibrosis and mediastinal fibrosis

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Immunoglobulin G4-related disease (IgG4-RD) is now a widely recognised multi-organ system disease characterised by elevated serum and tissue concentrations of IgG4. Two forms of thyroid involvement in IgG4-RD have been described, including Reidel's thyroiditis (IgG4-related thyroid disease) and the fibrous variant of Hashimoto's thyroiditis.
Objective
To describe a case of the development of massive neck fibrosis and mediastinal fibrosis in a patient with the fibrous variant Hashimoto's thyroiditis.
Methods and case presentation
In January 2012, a 59-year-old woman with a 1-year history of subclinical hypothyroidism due to Hashimoto's autoimmune thyroiditis was admitted to our department, presenting with a swelling in the anterior region of the neck, dyspnea, dysphagia, fatigue. The tumor progressed quickly to a ecklarge, stony hard mass on the anterior neck region. Analysis: TSH, 0.42 mU/ml, FT_4, 13.90 pmol/l;

AbTPO, 133.92 UI/ml, erythrocyte sedimentation rate (ESR), 58 mm/h; Echo-guided FNAB confirmed the diagnosis of Hashimoto thyroiditis and neck fibrosis. A computed tomography (CT) revealed a fibrotic mass located at the anterior-inferior aspect of the neck and mediastinum with displacement of the tracheal lumen and stenosis of oesophagus. We started therapy with prednisolone 60 mg daily and L-thyroxine substitution therapy. The follow-up lasted for 12 months. After 1 month of corticosteroid therapy, the patient had no compression symptoms. The treatment with prednisolone led to a significant subjective improvement and objective changes (a significant decrease of neck and mediastinal fibrosis), confirmed by regular clinical examinations, ultrasonography and computed tomography of the neck and mediastinum. Now the patient is in good health, undergoing a therapy by prednisolone 5 mg daily.

Conclusion

We have reported an unusual case of development of the massive neck fibrosis and mediastinal fibrosis in a patient with Hashimoto's thyroiditis. which showed good response to steroid treatment. It is important for the physician to recognize the presence of mediastinal fibrosis in the presence of fibrous variant of Hashimoto's Thyroiditis and initiate steroid therapy for resolution of both diseases.

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Differentiated thyroid cancer presenting as chylothorax

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Introduction

Initial presentation of thyroid carcinoma can range from a solitary thyroid nodule or cervical lymphadenopathy to symptoms related to local compression. Presentation as chylothorax due to compression of thoracic duct is exceptional. The aim of this case report is to describe an extremely rare case of follicular thyroid cancer presenting as chylothorax with special attention to therapeutic aspects.

Case report

A 78-years-old woman was referred to Internal Medicine Unit for evaluation of pleural effusion. She had a several months history of cough and dyspnea on exertion. Medical history was significant for hypertension and atrial fibrillation. The patient was tachypneic and the lung exam revealed markedly decreased breath sounds in the right hemithorax. Head and face examination were unremarkable. A computed tomography scan of the chest and neck showed a massive right pleural effusion and a markedly enlargement of right lobe of the thyroid gland with intrathoracic extension. Thoracentesis removed pleural fluid with the typical milky appearance of chylothorax. The diagnosis of chylothorax was confirmed by measuring the triglyceride levels of pleural fluid (469 mg/dl). A chest tube was placed in the right pleural space and parenteral nutrition was started. Fine-needle aspiration of thyroid mass was negative for malignancy. A transcervical approach to removal her substernal goiter was performed. A sternotomy was not required for removal and no thoracic duct repair or ligation was necessary. Biopsy of the thyroid mass was positive for follicular carcinoma without evidence of metastases. Chest tube was removed and oral nutrition was reinitiated. The patient received radioiodine ablation and postoperative follow-up showed no residual chylothorax.

Conclusions

It is rarely described the association of chylous pleural effusion and thyroid cancer. To the best of our knowledge this is the first case of follicular carcinoma presenting as chylothorax. The current case also highlights the potential surgical treatment of chylothorax associated to substernal goiter through transcervical approach without need of thoracic duct repair.

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A case of confirmed Smith–Lemli–Opitz syndrome

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Smith–Lemli–Opitz syndrome (SLOS) is a 46,XY disorder of sex development, included in the subgroup of disorders in androgene synthesis. The disease is caused by mutations of 7-dehydrocholesterol reductase (DHCR7) gene, conducting to deficient synthesis of the correspondent enzyme and of cholesterol, with important role in embryogenesis, adrenal and gonadal steroidogenesis. Clinical manifestations includ facial dysmorphism, syndactyly, ambiguous external genitalia and visceral malformations.

We present the case of an infant born by cesarean section for fetal suffering (Apgar score 8/1′–8/5′), with multiple malformations (dysmorphic face, polydactyly, syndactyly, hypospadias, cryptorchidism) detected already at birth. Investigations diagnosed small atrial septal defect and corpus callosum agenesis was suspected. The clinical examination at 4 months of age showed growth failure, dysmorphic face, bilateral ptosis and epicantus, generalized muscle hypotony, asymmetrical polydactyly (complete on right hand, partial on left hand), bilateral "Y" shaped syndactyly of the second and third toes, micropenis, hypospadias, small scrotum, cryptorchidism, neuro-reflexo-motorical developmental level of 1 month, psychical and verbal developmental level 2–3 months. Normal cortisol at baseline (17.18 μg/dl, *n*: 6.4–21), low total testosterone (0.74 ng/ml) and DHEA-S (3.1 μg/dl, *n*: 3.4–123.6), high LH (5.22 mIU/ml, *n*: 0.5–4) were measured. Cholesterol was reduced (total cholesterol 79 mg/dl, HDL-cholesterol: 9.2 mg/dl), and in context of dysmorphic facies and anomaly of external genitalia the suspicion of SLOS rises. The karyotype is 46,XY 1qh+. Mutation analysis of DHCR7 gene (chromosome 11q13.2–q13.5) identified two heterozygous mutations: c.452G>A (p.Trp151X) and c.278C>T (p. Thr93Met), this case being the first genetically confirmed SLOS in our country. An adequate diet, neurological recuperation, neurotrophic treatment, and endocrino-metabolic follow-up was recommended.

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A case of generalized resistance to thyroid hormone with chronic thyroiditis

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Introduction

Resistance to thyroid hormone (RTH) is a rare disorder characterized by reduced tissues's responsiveness to thyroid hormones (TH). Usually patients with RTH have not clinical signs except goiter. We describe a 70-years old woman with RTH and autoimmune thyroiditis simultaneously.

Case report

Woman 70-years old was hospitalized in our department on August 2011 with suspicious of Thyrotropinoma because of high levels of TSH and fT_4.The patient has atrial fibrillation controlled by the β-blockers since 2006. At that time only TSH was assessed – 6.8 mU/l (*n* 0.4–4.0) and L-T_4 50 μg/day was prescribed. After 3 months TSH decreased to 1.9 mU/l, L-T_4 was reduced to 25 μg/day. Such dose of L-T_4 she took for 3 years and discontinued by herself. Hormonal assessment after 1 year of discontinuation of L-T_4 (June 2011): TSH – 9.4 mU/l, fT_4 – 118.4 pmol/l (*n* 11–23), T_3 – 10.0 nmol/l (1.4–4.0), AbTPO – 515 U/l (0–30). Thyroid US revealed a goiter – 50 ml, inhomogeneous, without nodules. There were no any clinical symptoms of thyrotoxicosis except normosystolic atrial fibrillation (on β-blockers). We confirmed high levels of TSH and TH. Pituitary MRI was normal. We supposed that thyrotropinoma has not seen on MRI because of it's small size. Methimazole was prescribed (5 mg/d). In 1 month TSH increased to 33.8 mU/l, fT_4 decreased to 7.5 pmol/l (9–19.1) and methimazole was stopped, AbTPO increased to 1000 U/l. Next hospitalization of the patient was on April 2012. There were no signs of thyrotoxicosis, atrial fibrillation continued. The complaints on decreased of memory appeared. Thyroid volume was 52 ml, no nodules. TSH – 14.3 mU/l, fT_4 – 52.1 pmol/l (11–23), T_3 –14.7 nmol/l (1–2.8), thyroglobulin – 1.45 ng/ml (0–50). We did not reveal any metabolic disturbances, SHBG – 88.1 nmol/l (*n* 28–112), cholesterol – 5 mmol/l (3.3–5.2). Pituitary MRI with contrast was normal. FNAB of thyroid tissue have been done: picture of autoimmune thyroiditis revealed. The patient discharged from hospital with no any thyroid treatment, only on β-blockers and anticoagulants for atrial fibriilation. The patient is on our's observation now.

Conclusion

In this case, we have two different conditions: RTH and autoimmune thyroiditis. We supposed that patients will be hypothyroid in the future.

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Autoimmune hypothyroidism converted to hyperthyroidism: is it a common phenomenon?

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Introduction
Graves' disease and Hashimoto's thyroiditis are the two autoimmune spectrum of thyroid disease. Cases of conversion from hyperthyroidism to hypothyroidism have been reported but conversion from hypothyroidism to hyperthyroidism is very rare although reported. We report a case of hypothyroidism that converted to a hyperthyroid state needing treatment.
Case report
A 36 years old female presented with a 3 months history of easy fatigability, cold intolerance, polymenorrhagia, constipation and weight gain. On examination she hadbradycardia and dry skin. Thyroid gland showed small diffuse enlargement. Clinical suspicion of primary hypothyroidism was made and thenconfirmed by TSH of >50 uIU/ml (0.4–4.2 uIU/ml) with FT_4 value of <0.30 ng/dl and positive thyroid antibody titre. Thyroxine was started at 100 µg/day. Gradually requirement of thyroxine decreased and she maintained her TSH within normal range on 50 µg/day of thyroxine. After 3 years dose was further reduced to 25 µg/day but after 2 years again thyroxine dose was increased to 50 µg/day because of slightly increased TSH of 8.86. Slightly more than a year later she presented with weight loss of 3 kg and feeling of anxiety and tremors of hands. TSH was <0.005 with a FT_4 of 2.4 confirming the state of thyrotoxicosis. Thyroxine was stopped and patient was observed over a period of 6 months. She remained clinically and biochemically hyperthyroid with a repeat TSH of <0.005 and FT_4 of 2.66. Thyroid scintigraphy showed an increased homogenous tracer uptake. She was started on Carbimazoleand she remains on it till date.
Conclusion
This case demonstrate that high index of suspicion should be there if a patient with primary hypothyroidism develop persistent symptoms of hyperthyroidism. Otherwise it can be missed easily considering it as an over replacement with thyroid hormone.

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Maturity onset diabetes of youth (MODY) in a patient with VATER syndrome

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Introduction
VATER syndrome is a rare, usually sporadic, entity, including ≥ 2 of the following: vertebral defects (V), anal atresia (A), tracheoesophegeal fistula, esophageal atresia (TE) and radial or renal dysplasia (R).
Maturity onset diabetes of youth (MODY) is a usually non-insulin-dependent type of monogenic diabetes mellitus (DM), usually in adults <25 years, with autosomal dominant inheritance, without the features of metabolic syndrome.
Case report
A 25-year-old Caucasian male with VATER syndrome was admitted to our department due to newly diagnosed DM on occasion of polydepsia and polyuria, without ketoacidosis (fasting plasma glucose (FPG) levels: 355 mg/dl, glycosylated hemoglobin (HbA1c): 12.6%). His family history was positive for non-insulin dependent DM (diagnosed at 35 years).
Clinical examination was remarkable for a low body mass index (18.28 kg/m^2), muscular weakness of the extremities and hearing loss.
Investigation for autoantibodies to glutamic acid decarboxylase (GAD), insulin, islet cells and tyrosine phosphatase (IA2) was negative.
During an oral-glucose-tolerance-test (OGTT), FPG, fasting insulin and C-peptide levels at baseline were: 150 mg/dl, 2 mIU/ml (normal: 6–27) and 1.07 ng/ml (normal: 0.9–7.1), and 2 h after glucose 75 g: 256 mg/dl, 11.7 mIU/ml and 3.38 ng/ml, respectively.
He was treated with glimepiride 2 mg/day. At 3 months, HbA1c fell to 5.9%. He remained on glimepiride for 1 year, when due to glucose control deterioration, insulin was started.
He fulfils the five criteria of MODY: early-age newly DM with normal insulin and C-peptide levels, positive family history for DM, >90 mg/dl increase in plasma glucose during OGTT, glycosuria with relatively normal FPG and responsiveness to sulfonylureas. Genetic analysis for MODY 3 (the most common) was negative. Further testing to rule out mitochondrial diabetes is under way.
Conclusions
This is the first case reporting the co-existence of MODY with VATER syndrome. The exact pathogenetic mechanisms linking these entities are currently unknown.

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Plasmapheresis in rapid preparation of a patient with toxic multinodular goiter for surgery

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Thyroidectomy is the definitive treatment for toxic multinodular goiter (TXMNG). Surgery should be performed when the patient is euthyroid which decreases perioperative cardiac risks. Plasmapheresis is a procedure that removes the thyroid hormones from the circulation. It is an alternative method in case of resistance or contraindications to anti thyroid drugs. Role of plasmapheresis in the treatment of TXMNG is not fully determined in the literature. We report a case with TXMNG who was prepared for surgery by plasmapheresis due to side effects of antithyroid drugs.
A 54-year-old female diagnosed as TXMNG was admitted to Sisli Etfal Training and Research Hospital, Endocrinology Clinics because of a complicated hyperthyroidism. While on propylthiouracil (PTU) therapy, she exhibited erythematous, tender nodules and plaques at bilateral lower extremities. PTU was stopped. Propylthiouracil related ANCA(+) panniculitis was diagnosed based on biopsy and laboratory findings. On the 7th day of systemic steroid therapy, signs and symptoms of hyperthyroidism recurred (TSH:0.005 uIU/ml, fT_4:7.77 ng/dl, fT_3:9.49 pg/ml). Ultrasonographic examination revealed a diffusely enlarged thyroid gland with multiple nodules, right lobe extending retrosternally. Thyroid scintigraphy with 99 m technetium showed a hypoactive nodule in the left lobe and a hypoactive nodule with a hyperactive component in the right lobe. Rest of the thyroid other than nodules showed high activity uptake. Methymazole 40 mg/day was started along with β-blocker. As euthyroidism could not be achieved on the 3rd week, plasmapheresis was decided for rapid preparation to surgery. The patient had seven sessions of plasmapheresis performed with filtration method (Infomed, CS-220, Switzerland). The replacement fluid was ten fresh frozen plasma for each session (totally 2000 cc). Although thyroid hormone levels could only moderately decreased, the patient was asymptomatic. The patient underwent surgery with β-blocker and dexamethasone treatment to avoid the thyroid storm in the perioperative period. Total thyroidectomy was performed successfully and adenomatous nodular hyperplasia was diagnosed pathologically.
Plasmapheresis can be considered a safe and effective alternative procedure to prepare patients with TXMNG for surgery when drug treatment fails or is contraindicated.

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Postmenopausal hirsutism and hyperandrogenemia due to granulosa cell tumor of the ovary

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Inroduction
Progressive baldness and severe hirsutism associated with granulosa cell tumor of ovaries are rare conditions in women. Reported cases are usually pubertal girls and young women. Here, we report a case of postmenopausal baldness and hirsutism due to granulosa cell tumor of the ovary.

Case report
A 62 year-old woman presented with baldness and hirsutism on her face, body and back for 3 years. She had menarche at 14 years and menopause at 52 years. She had regular menstrual periods until menopause. She had history of infertility which was refractory to treatment and impaired fasting glucose. Four years ago, she had undergone left oopherectomy due to an ovarian cyst which was benign in histological examination. On physical examination, Ferriman–Gallwey index was 24 with severe hirsutism on her face, body and back and male pattern baldness on her scalp. She was normotensive, her body mass index was 34 kg/m^2 and there was no cliteromegaly. Hormonal profile revealed elevated total testosterone, 5.31 ng/ml (0.06–0.82) and free testosterone, 18 pg/ml (0.29–3.18), low FSH 2.35 mIU/ml (25.8–134.8) and LH 3.45 mIU/ml (7.7–59) for postmenopausal women. Estradiol, prolactin, dehydroepiandrosterone sulphate, 17-hydroxyprogesterone, TSH and IGF1 levels were normal. She had 46, XX karyotype. Magnetic resonance imaging of abdomen and pelvis revealed a right ovarian mass. She underwent laparoscopically-assisted hysterectomy and right salpingo-oophorectomy. Microscopic examination of the right ovarian tumor revealed granulosa cell tumor. Three months after surgery, total and free testosterone levels decreased to 0.121 ng/ml and 0.68 pg/ml, respectively. FSH and LH rised to 25.76 and 21.31 mIU/ml, respectively. At 9 months, her testosterone levels were still low and hirsutism and baldness improved.
Conclusions
Although granulosa cell tumors are generally associated with manifestations of hyperestrogenism, our patient was in hyperandrogenemic and improved both clinically and biochemically after the operation.

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Clinical case report: male patient with SRY-positive 46,XX testicular disorder of sex development

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The 46 XX male syndrome (de la Chapelle syndrome or 46,XX testicular disorder of sex development -46,XX testicular DSD-) is a rare phenotype associated with disorder of the sex chromosomes. XX males exist in different clinical categories with ambiguous genitalia or partially to fully mature male genitalia, in combination with complete or incomplete masculinization.
Clinical report
We describe the clinical, molecular, and cytogenetic findings of a 26-year-old male patient with 46,XX testicular DSD.
The patient attended our endocrinology clinic with complaints of gynecomastia.. His height was 165 cm and weight 76.6 kg (BMI:28.13 kg/m^2). He presented bilateral gynecomastia for the last 7 years. At presentation, hormonal laboratory evaluation revealed elevated serum concentrations of FSH and LH, with low concentrations of Testosterone (230 ng/dl). The testicles were descended in the scrotum but small in size with volumes 3.5 and 4 ml (normal range 18–30 ml). Axillary and pubic hairs were of normal pattern but low density.
Testicular ultrasound revealed testes hypotrophia (right testes: 23.8×14.4×12.5 mm, left testes: 24.7×10.3×16.3 mm)without nodes or calcium intratestes. Abdominal pelvic ultrasonography (USG) showed normal seminal vesicles and prostate and no mullerian derivatives.
Semen analysis showed azoospermia.
Chromosomal analysis revealed 46,XX karyotype. Fluorescence *in situ* hybridization (FISH) showed the SRY region translocated to the short arm of the X chromosome. The presence of the SRY gene was also confirmed by polymerase chain reaction (PCR).
Conclusion
The 46,XX testicular DSD is a rare form of sex reversal with complex mechanisms leading to a large spectrum of clinical manifestations ranging from ambiguous genitalia in the newborn to normal male phenotype. Therefore, diagnosis is established either pre- or early postnatal, or in adult life due to male infertility or as in our case, due to gynecomastia.
Our patient has a 46,XX Karyotype, normal male phenotype and hypergonadotropic hipogonadism leading to infertility.

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P289

Insulin autoimmune syndrome, IAS (Hirata disease) case report

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Introduction
Insulin autoimmune syndrome was first described in 1973 by Hirata, characterised by recurrent spontaneous postprandial hypoglycaemia. Serum insulin is extremely high with elevated insulin autoantibodies. More than 170 cases reported worldwide. We report the first case of IAS in Australia.
Case report
Eighty one-year old Argentinean woman with 3 month history of intermittent, late postprandial diaphoresis, tremors, palpitations, dizziness and confusion. Symptoms improved with sugary drinks and other caloric intake resulting in weight gain of 3 kg. At presentation blood glucose level (BGL) was 1.2 mmol/l and required 50% dextrose to maintain her BGL >6. Serum insulin was >2400 mU/l (normal <27), C-peptide 11.7 nmol/l (0.4–1.5) and sulphonylurea screening was negative. Abdominal CT, MRI and Dotatate PET scan failed to identify an insulinoma. Endoscopic ultrasound found an 8 mm pancreatic lesion. Calcium stimulation study showed high insulin without a gradient. Laparotomy failed to show an insulinoma. Diagnosis of Hirata disease with elevated insulin autoantibodies >50 U/l (<0.3) was treated with reducing prednisone dosage maintaining remission.
Discussion
Mechanism of IAS is that antibodies against insulin binds and releases insulin asynchronously. Free insulin levels are normal though bound (total) are high. Incidence of IAS in Caucasians is one-tenth of Japanese. Eighty percent of IAS patients spontaneously remit. In some individuals it is associated with exposure to sulfhydryl compounds interacting with disulphide bonds rendering insulin more immunogenic. IAS is strongly associated with HLA class II alleles. Insulinomas are the most prevalent cause of hyperinsulinemic hypoglycaemia in Caucasians. Investigation is focused on localization followed by surgery. Our case highlights important differential diagnosis, the need to measure insulin antibodies to avoid unnecessary surgery.

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Neurogenic hypertension as a case report

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Introduction
Neurogenic hypertension is associated with unilateral neurovascular compression of the brainstem and cranial nerves V, IX-X, VII, VIII. Causes may include nerve injury, vascular compression, tumors. Symptomatology seems hyperactive dysfunction syndrome of the cranial nerves. In the clinical picture, it can be seen trigeminal neuralgia, vertigo, tinnitus, hemifacial spasm, and in some cases hypertension.
Case report
Female patient, 45 years old, pianist, hospitalized in our department to investigate the etiology of hypertension. Symptoms began two years ago, while working at the computer, in the form of a sudden attack of vertigo, disorientation, anxious, with the simultaneous appearance of facial asymmetry (swollen left eyelid and down, left eyebrow and left corner of the mouth), and pain in the left side of the head, followed by high pressure 170/100 mmHg and tachycardia 120/min. The attack lasted about 20 min and passed spontaneously. Such attacks were two to three times a month and sometimes weekly. Between attacks she was healthy. The results of the functional and morphological studies show that the causes of the attacks are pathological changes in the pons. Examinations excluded renovascular and endocrine causes of hypertension. Magnetic resonance imaging of the head found a malformation of blood vessels in the border edge infratentorially midbrain and pons. Electroencephalogram was normal several times.
Conclusion
Neurogenic hypertension was the result of vascular compression of the cranial nerves and brainstem and treatment with Bisoprolol and Tegretol led to a reduction in the frequency of attacks.

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P291

Immobilisation hypercalcaemia in a young man

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Background
Immobilisation hypercalcaemia is serious complication of prolonged immobility of any cause such as spinal cord injury, polio victims, burns victims, as well as trauma patients.
Case
Eighteen-years old student was admitted to the hospital following road traffic accident. He sustained multiple fractures, skull, spine, chest and pelvis. He also sustained abdomen and pelvic haematoma. At scene of crash he had GCS 5/15, needing intubation and was aiirlifted to trauma centre, where he underwent extensive surgery laparatomy and pre-peritoneal packing, reduction of fracture dislocation of right, open reduction and fixation of bilateral pelvics fracture and acetabulum. His past medical includes orchidectomy after failed orchidopexy, and epistaxisis. After that he was transferred to his local Hospital for rehabilitation. His bloods showed Na^+ 143, K 3.3, creatinine 45, calcium 3.01, PTH 0.9 pmol/l, 24 h urinary calcium 15.1 mmol/l vitamin D 46 phosphate 1.08, ALP 95, AST 45, Hb 12. He had normal calcium (2.25 mmol/l) during his initial admission to the trauma centre.
He was treated with i.v. fluids and pamidronate 30 mg single dose and his calcium level normalise. His calcium remains normal and the patient is undergoing physiotherapy.
Discussion
Immobilisation hypercalcaemia is a common complication of prolonged immobility of different causes including road traffic accident. Although well described among young patients with spinal cord it may happen in immobile patients following road traffic accident as demonstrated in our case report. It usually develops 1–16 weeks post trauma, and it may remain elevated up to 12 months.
Conclusion
Prolonged immobility following road traffic accident may cause hypercalcaemia which is reversible.

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Parathyroid carcinoma: an atypical case in a patient submitted to Bariatric surgery

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Background
Parathyroid carcinoma accounts for $<1\%$ of cases of primary hyperparathyroidism. Clinical presentation is usually related to severe hypercalcemia associated to elevated serum PTH (three times above the upper limit). These values are so much higher than in primary hyperparathyroidism due to a benign adenoma. Moreover, 30–75% of patients had a palpable neck mass.
Clinical case
Fifty one-year-old male, submitted to bariatric surgery by a biliopancreatic diversion (Larrad's technique) in 2002. He left treatment and follow-up at Nutrition Department. He is admitted to hospital because of an acute respiratory failure when a right cervical mass is discovered. TC showed a trachea deviation related to a 7 cm mass depending from the right thyroid lobe. FNA was suspicious for a follicular neoplasm with Hürthle cells. In previous analysis patient had many normal calcium values but some slightly elevated, chronic hypophosphatemia and very high intact PTH (values above 500 pg/ml, range 9–70). Surgery was performed including a total thyroidectomy, cervical exploration looking for any parathyroid adenoma and a tracheostomy. Histological study revealed a parathyroid carcinoma of $7.8\ cm \times 4.6\ cm \times 3.3\ cm$ and one-gland hyperplasia. Patient developed a hungry bone syndrome with high needs of calcium and calcitriol. 25OHvitamin D was undetectable and post-surgical PTH was 178.5 pg/ml.
Conclusion
Main form of presentation of parathyroid carcinoma is severe hypercalcemia. However, our patient had a normocalcemic hyperparathyroidism. Absence of hypercalcemia despite of so high PTH is explained by a malabsortive bariatric procedure that excluded duodenum and first part of jejunum conditioning a chronic malabsortion of calcium and vitamin D.

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Autoimmune polyglandular syndrome type IV with twins pregnancy: clinical, diagnostic, evolutive aspects and treatment

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Autoimmune polyglandular syndromes (APS) are characterized by the association of two or more endocrine and/or nonendocrine autoimmune diseases with different immunologic features of their pathogenesis.
Based on the clinical picture is divided into four different types. Type I APS comprises mucocutaneous candidiasis, hypoparathyroidism and Addison's disease. Type II APS is defined by autoimmune Addison's disease in association with chronic autoimmune thyroiditis and/or type one diabetes mellitus. Type III APS is composed of autoimmune thyroid diseases associated with other autoimmune conditions, excepting Addison's disease. The rest of autoimmune combinations not included in the previous groups, belong to type IV APS.
We present a 40 years old woman who was diagnosed with chronic autoimmune thyroiditis and Addison's disease four years ago, with subsequent development of hipoparathyroidism (possibly autoimmune mechanism?). At age 37, she became first time pregnant with twins. We discuss clinical, diagnostic, evolutive aspects and especially those of treatments during pregnancy, taking into consideration the number of co morbidities who requiring carefully medication with possible drug interactions and adverse effects.

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P294

Hypercalcemia in patient five years after the diagnosis of gastrinoma

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Introduction
We diagnosed multiple endocrine neoplasia type 1 (MEN1) based upon the occurrence of two primary MEN1 tumor types in patient without family history of MEN1. Hyperparathyroidism was diagnosed in patient five years after gastrinoma surgery. With hormone tests and morphologic investigation we excluded pituitary adenoma.
Case report
Forty-nine year old patient was admitted with abdominal pain and vomiting. Laboratory testing revealed elevated values of calcium 3.29 mmol/l (normal range 2.2–2.6 mmol/l), ionized-calcium 1.88 mmol/l (1.12–1.23 mmol/l) and intact parathyroid hormone 365 ng/l (10–65 ng/l). We performed ultrasonography of neck region and subtraction thyroid scan. Investigations showed multiple parathyroid adenomas. The patient was referred to thoracic surgeon. Two years ago, he was diagnosed whit kidney stones and was not referred to endocrinologist. In 2007, he was diagnosed with gastrinoma. Tumor was located in pancreatic head, there was no metastatic disease. Whipple operation was performed. At that point we excluded the possibility of MEN1 with laboratory testing.
Discussion
MEN1 is heritable disorder characterized by a predisposition to parathyroid adenomas, anterior pituitary adenomas and tumors of pancreatic islet cells. Multiple parathyroid adenomas causing hyperparathyroidism are the most common manifestation of MEN1 with almost 100% penetrance and in the most cases the initial manifestation. Pancreatis islet cell and gastrointestinal adenomas become clinically apparent in one-third of patient, approximately 60% have Zollinger–Ellison syndrome (ZES). ZES is initial clinical manifestation of MEN1 in 40% of patient. On the other hand, MEN1 is present in 20–60% of patients with ZES. Pituitary disease occurred in 60% of patients.
DNA testing for MEN1 gene mutations is available and can provide valuable information in specific situation.
Conclusion
Annually monitoring and biocemical screening in all patients with diagnosed MEN1-associated tumors has to be considered.

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Presence of Paget's disease in a patient with endometrium carcinoma

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Introduction
Isolated bone metastasis of endometrium carcinoma is rare; but if it occurs, pelvis and vertebrae are involved mostly. Co-existence of undiagnosed Paget's disease in a patient with malignancy causes major problems in differential diagnosis.
Case
A 77 year-old woman was admitted to our hospital with severe pelvic pain. She had been diagnosed with inoperable endometrium adenocarcinoma for over a year. Metastatic bone disease was suspected because of pain localized to pelvic region. Sacroiliac graphy and computed tomography (CT) indicated lytic lesions on sacrum, pubic ischiadicum and L5 vertebra. Increased uptake was evident in bone scintigram (Tc99). These lesions were reported as metastatic bone involvement. However, Paget's disease was also considered in differential diagnosis. In (18)F-fluorodeoxyglucose positron emission tomography/CT [(18) F-FDG PET/CT], osteolytic and osteosclerotic lesion areas showed heterogenous FDG uptake in early (SUV_{max}:7.4) and late images (SUV_{max}:8.7), also fused images showed moderate FDG uptake on osteosclerotic lesions (SUV_{max}:3.7) which may be compatible with Paget's disease. She was referred to Endocrinology Department with a suspect of Paget's disease. Bone biopsy which was performed to exclude metastatic carcinoma revealed osteoblastic and osteoclastic activity in trabecular bone attributable to Paget's disease and no focus of tumor infiltration was evident. Laboratory evaluations were as follows: BUN:24 mg/dl (*n*: 8–22 mg/dl), creatinine: 2.3 mg/dl (*n*: 0.7–1.4 mg/dl), serum corrected calcium: 9.6 mg/dl, PTH: 80 pg/ml (*n*: 15–65 pg/ml), β-CTX: 1.2 ng/ml (<1 ng/ml) ALP: 120 U/l (*n*: 35–105 U/l) Calcitonin (s.c.) was applied to control the disease as the patient's glomerular filtration rate was 24.3 ml/min per 1.73 m^2.
Conclusion
Our patient had active disease and showed low-moderate uptake (lower than expected in malignant disease). If osteoblastic and osteoclastic activity of Paget's disease is associated with increased glycolysis and glucose use, it could cause false-positive uptake of (18F)FDG in PET studies. Paget's disease should be considered in differential diagnosis of positive bone scintigraphy finding during screening for bone metastasis.

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Parathyroid carcinoma

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Introduction
Parathyroid carcinoma is a rare endocrine malignancy accounting for <1% of all cases of hyperparathyroidism. Diagnosis and treatment is still difficult.
Case report
A 32-year-old women was admitted to the hospital because of mental status changes, polyuria, polydipsia, musculoskeletal pain and weight loss (34 kg during last year). She had history of nephrolithiasis, pancreatitis, cholelithiasis, peptic ulcer disease, osteoporosis and diabetes. She underwent right lower parathyroidectomy (histopathology: nodular hyperplasia) 3 years before reported hospitalization. On physical examination: neck exam showed a 4 cm, hard, nodular mass in the anterior neck with lymphadenopathy. Routine blood tests revealed severe hypercalcemia (serum calcium of 4.14 (mmol/l)), elevated intact PTH (2374 pg/ml) and alkaline phosphatase (600 UI/l). Ultrasound of the neck showed enlargement lymph nodes and hypoechoic mass below of the right lower thyroid lobe (1.9×2,2 cm) suggestive of a solid nodule of the right lower parathyroid. PET scan showed additional metastases to the lymph nodes and lungs. Parathyroid carcinoma was diagnosed on histopathology after cytoreduction surgery. She underwent radiotherapy after surgery and received chemotherapy.
Conclusion
Parathyroid carcinoma is highly suggestive in young age, palpable neck mass, concomitant renal, pancreas, skeletal disease and extremely high iPTH level.

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Primary hyperparathyroidism associated with atrial septal defect, interatrial septal aneurisym and skeletal anomaly: a case report

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The classic clinic manifestation of primary hyperparathyroidism (PHPT) is osteitis fibrosa cystica, a severe skeletal disease characterized by brown tumors, bone cysts and deformities, due to extremely elevated bone resorption elicited by continuously high parathyroid hormone (PTH) levels. In cardiovascular system there may be shortened QT interval, deposition of calcium in heart valves, coronary arteries, and myocardial fibers. Here, we report a case with PHPT, who have anorexia nervosa, skeletal anomaly, atrial septal defect (ASD) and interatrial septal aneurysm.
Case report
An 18-year-old woman, who has anorexia nervosa has applied for constipation. She had bradycardia, low weight (BMI: 15 kg/m^2), and prognathism. The radiographic finding of the extremities showed that, bilaterally 2 and 5 metacarpals were short in the hands, and bilaterally 1, 3, 4, 5 metatarsals in the foots were short. Laboratory tests revealed hypercalcemia, hypophosphatemia and hyperparathyroidism. Her 24-h urinary calcium was 504 mg/day. The neck ultrasound revealed a 4.9×6.8×10.4 mm hypoechoic lesion in the outside the thyroid right lobe inferior. Sestamibi scintigraphy results were consistent with parathyroid adenoma. Renal ultrasonography determined milimetric crystaloids in the right kidney. Bone mineral densitometry revealed osteopenia. Her electrocardiography showed a shortened QT interval and bradycardia. Echography revealed an ASD and interatrial septal aneurysm. Her pituitary hormonal levels were consistent with panhypopituitarysm. She had also evaluated for the eye, ear and nose anomaly, but no pathology was found. After the diagnosis and emergency teratment for hypercalcemia she had underwent parathyroidectomy. Postoperative her calcium and parathyroid levels were reduced in normal levels.
Conclusion
Our case revealed hypercalcemia and hyperparathyroidism and also unusual manifestations like anorexia, ASD, interatrial septal aneurysm and skeletal anomaly. In literature to our knowledge, there is only one case with PHPT and ASD associated with mongoloid features. In conclusion, the patients with PHPT may be evaluated for atypical manifestations of cardiac and skeletal system.

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Hypercalciuria in a patient with central diabetes insipidus

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Central diabetes insipidus is characterized by increased water excretion which is corrected by the administration of antidiuretic hormone. Hypercalciuria is known to be associated with osteoporosis. The aim was to describe the case of a patient with central diabetes insipidus, hypercalciuria, vitamin D deficiency and severe osteoporosis.
The case of a patient, female aged 64 years, presenting with central diabetes insipidus, hypercalciuria, vitamin D deficiency and severe osteoporosis is described. The patient suffered from central diabetes insipidus since the age of 20 years. Laboratory investigations revealed severe hypercalciuria, urine calcium levels being 800 mg/24 h, decreased blood calcium and vitamin D deficiency, $25(OH)D_3$ levels being 10 ng/ml (normal levels >30 ng/ml). PTH levels were increased and *T* score was −4. Vitamin D was administered along with a thiazide diuretic for the correction of vitamin D deficiency and hypercalciuria. Vitamin D levels increased and hypercalciuria was partially corrected. Sequentially, strontium ranelate was administered without an improvement in bone density. Strontium ranelate was stopped, alendronate was administered and bone mineral density increased.
Conclusions
The extremely rare case of a patient with central diabetes insipidus, hypercalciuria, vitamin D deficiency and severe osteoporosis is described. Hypercalciuria was partially resistant to thiazide therapy as well as osteoporosis to strontium ranelate, finally improving with bisphosphonates. The coexistence of hypercalciuria, salt losing nephropathy and renal diabetes insipidus has been described in the context of glomerular disease. Osteoporosis in the context of central diabetes insipidus has also been described, responding to bisphosphonates.

However, the coexistence of central diabetes insipidus with hypercalciuria and severe osteoporosis is extremely rare.

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A recurrent case of subacute thyroiditis improved with the onset of pregnancy

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Introduction
Thyroid disorders such as subacute thyroiditis occur rarely in pregnancy. We report a recurrent case of subacute thyroiditis improved with the onset of pregnancy.
Case report
A 32-year-old Turkish women who request pregnancy and planned insemination was referred due to malaise and neck pain in the left side a week ago. She was presented with low-grade fever, fatigue, pharingitis symptoms, neck pain and extremely tenderness in the right side of thyroid about four months ago. Subacute thyroiditis was diagnosed with colour doppler sonography of thyroid showed decreased vascularise and flow in the thyroid and elevated markers of inflammation. Owing to increasing symptoms and signs, glucocorticoid therapy was given. Three months after discontinuation of steroid therapy with relief, she was admitted to the other side neck pain and tenderness during the induction of ovulation. Her symptoms, signs and inflammatory parameters were progressed. Hence, steroid therapy was given a short course to the patient on five days before insemination. It is discontinued before the day of insemination. During first month of pregnancy, the clinic situation was resolved, and improved without need for medications. Because of hypothyroidism, levothyroxine was given throughout pregnancy.
Conclusions
Subacute thyroiditis is rare and an important disorders during pregnancy. The beginning of pregnancy, physiological and immunological mechanisms may be responsible for the recovery of subacute thyroiditis.

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P300

High rate of malignant disorders in patients with primary hyperparathyroidism

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Introduction
Some new data suggest a coincidence of differentiated thyroid carcinoma in patients with primary hyperparathyroidism (pHPT).
Methods & design
We performed a retrospective analysis of our patients ($n=267$) with primary hyperparathyroidism from 2007 until 2011.
Results
Musculoskeletal pain was reported by 34.2% of the patients. Gastrointestinal discomfort was complained by 28.8%, and 21.1% had nephrolithiasis. Out of 267 patients, 223 patients (174 women, 49 men) underwent parathyroidectomy. Fourty-four patients received conservative therapy. In 257 patients ultrasound examination showed uninodular goiter in 38 patients (14.8%) and multinodular goiter was found in 117 patients (45.5%). After surgery, histological specimens from thyroid glands revealed differentiated thyroid cancer in 17 patients (7.62%). Follicular thyroid cancer was seen in two patients (0.90%), papillary thyroid cancer in 14 patients (6.27%), and medullary thyroid carcinoma in one patient (0.45%). Eight patients had C-cell hyperplasia. Including the history of other malignant diseases, 26.1% of the patients had an oncologic disease. Of 223 patients, only 25 patients (11.2%) had no other disease than pHPT. Ninety-one patients (40.8%) suffered from endocrine diseases: multiple endocrine neoplasia type 1: 11 (4.93%), Hashimoto's thyroiditis: 42 (18.8%), Grave's disease: 1 (0.45%), Diabetes mellitus type 1 and type 2: 13 (5.8%), polyglandular autoimmune syndrome: 1 (0.5%). One hundred and seventy-four patients (78%) suffered from cardiovascular disease (mostly arterial hypertension), renal disease was found in 66 patients (29.6%) and 81 patients (36.3%) had gastrointestinal disease.
Conclusion
Thyroid cancer is not rare in patients with pHPT. Many of the patients have a history of other malignant diseases. Hashimoto's thyroiditis is a frequent thyroid disease in this group of patients. It still has to be investigated, if there is a causal relationship between malignancy and hyperparathyroidism.

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P301

Experience in the use of Tolvaptan in elderly patients with significant hyponatraemia

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Introduction
Tolvaptan is an oral vasopressin V_2 receptor antagonist which offers a novel treatment for euvolaemic and hypervolaemic hyponatraemia. Here, we report our experience with Tolvaptan in elderly patients.
Case 1
Seventy sex-year-old lady with background of hypothyroidism, hypertension and alcohol excess presented with acute onset of confusion. Her admission Sodium [Na^+] level was 117 mmol/l and represented an acute drop from normal level after a thiazide diuretic was introduced two days earlier. Acute thyroid dysfunction and adrenal insufficiency were excluded. Despite stopping bendroflumethiazide, [Na^+] fell further to 108 mmol/l 48 h later. Urinary spot [Na^+] was 29 mmol/l and urine osmolality 766 mOsm/kg in the context of euvolaemia. Following administration of 15 mg of Tolvaptan, [Na^+] level rose to 117 mmol/l on day one and 122 mmol/l on day 2. Tolvaptan was discontinued and hyponatraemia improved on fluid restriction only with [Na^+] level 132 mml/l 48 h later.
Case 2
Ninety one-year-old lady with known congestive cardiac failure and hypertension was admitted with a fall. She was known to have mild hyponatraemia, secondary to loop diuretic use. [Na^+] level fell rapidly from 130 to 115 mmol/l a week post admission and continued to decline despite withholding the diuretic and ACEi, fluid restriction and Demeclocycline use (300 mg 6 hourly). Plasma osmolality was low at 245 mOsm/kg with urine osmolality at 598 mOsm/kg. Thyroid dysfunction and hypocortisolaemia were excluded. Tolvaptan 15 mg was introduced at [Na^+] level of 106 mmol/l and resulted in gradual improvement in hyponatraemia with [Na^+] level at 111 mmol/l on day 1, 118 mmol/l on day 2 and 127 mmo/l on day 3 post Tolvaptan. The medication was discontinued and [Na^+] level remained stable on fluid restriction.
Conclusion
Tolvaptan is a safe and effective treatment of hyponatraemia in elderly population providing more prompt rise in serum sodium than fluid restriction and Demeclocycline.

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Hypercalcemia secondary to concomitant thyrotoxicosis and B cell lymphoma

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Introduction
Mild hypercalcaemia can present in 10–15% of hyperthyroid patients. In contrast, its incidence in B cell lymphoma is considerably less (7–8%) but its levels are much higher. We report a patient with hypercalcemia secondary to both thyrotoxicosis and lymphoma.
Case report
A 55 year-old lady presented with non specific symptoms of appetite loss and lower limb weakness. Comorbidities included hypertension, diabetes mellitus, hyperlipidaemia and a previous history of consumption of traditional herbal supplements. Examination was unremarkable except for bilateral hand tremors.

There was no hepatosplenomegaly or lymphadenopathy. Laboratory results: Ca(adj) 3.59 mmol/l (RI: 2.15–2.58), PO4 1.2 mmol/l (RI: 0.8–1.6), PTH < 0.6 pmol/l, 25(OH)VitD 19 ug/l (RI: 30–50). fT_4 67 pmol/l (RI: 8–21), TSH 0.03 mIU/l (RI: 0.34–5.6). TRAb 7.5 IU/l. A normal short synacthen test excluded concomitant hypocortisolism. A presumptive diagnosis of PTH-independent hypercalcaemia due to thyrotoxicosis was made. A dose of pamidronate normalised the calcium levels but despite treatment with anti-thyroid medications, hypercalcemia rapidly recurred. A search for malignancy revealed elevated LDH and beta2 microglobulin and multiple lymphadenopathy on abdominal imaging. This was followed up with a bone marrow examination showing B cell lymphoma. She was subsequently referred to hematology for chemotherapy.

Conclusion

Despite the presence of a known cause of hypercalcemia, malignancy must always be extensively investigated for and excluded when calcium levels are markedly elevated. Hypercalcemia in B-cell lymphoma is not uncommon and carries a poorer prognosis, thus it is crucial that diagnosis be suspected and made early so that appropriate treatment can be carried out.

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P303

Hyperthyroidism-induced heart block: a case series in the Philippine general hospital

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Cardiac arrhythmias in thyrotoxicosis are usually seen as sinus tachycardia and atrial fibrillation but conduction abnormalities in the form of heart blocks do occur in rare instances. We present here four hyperthyroid patients with complete heart blocks. Cases 1 and 2 are young women diagnosed with Graves' disease who both experienced syncope and were seen to have 3rd degree atrio-ventricular block. Case 3 is a young woman who presented with palpitations, fever and shortness of breath. Her 12 lead ECG showed diffuse ST-segment elevation consistent with pericarditis and complete heart block. Case 4 is a young male with enlarged anterior neck mass and hyperthyroid symptoms who was not cleared for thyroidectomy due to 3rd degree AV block. Development of conduction problems in hyperthyroidism can be due to overwhelming thyroid hormones per se and contributed by acute infections, use of rate control drugs, electrolyte imbalances, and cardiac anomalies. Resolution of clinical, electrophysiological, and biochemical abnormalities occurred in our patients after achieving euthyroidism.

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P304

A case with thyroid acropachy as the initial manifestation of thyrotoxicosis

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Thyroid acropachy is an uncommon and usually late complication of thyroid disease. The typical clinical presentation of thyroid acropachy includes clubbing, distal soft tissue swelling and periosteal reaction involving the tubular bones of the hands and feet. It is usually associated with exophthalmos and thyroid dermopathy. We present a case with thyroid acropachy as an initial manifestation of toxic adenoma.

A 69-year-old man was admitted to our out-patient clinic with complaints of pain and swelling in his hands and wrists. These symptoms had been present for 3 years. His physical examination revealed clubbing of the fingers of both hands and exophthalmos. Dermopathy was not observed in our patient. Radiographs of both hands revealed periosteal reaction that was bilateral and symmetrical involving all proximal phalanxes. Laboratory investigation showed elevated serum T_3, depressed TSH levels. Serum T_3 and alkaline phosphotase levels were in normal range. Thyroid scintigraphy demonstrated a toxic adenoma in the right lobe of thyroid gland. The patient was diagnosed as thyrotoxicosis with thyroid acropachy and medical treatment was initiated involving antithyroid drugs and low dose steroid.

We present this case with thyroid acropachy as an unusual initial presentation of thyrotoxicosis.

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P305

Postmenopausal Sertoli–Leydig cell tumor in two patients

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Introduction

Sertoli–Leydig cell tumours are rare tumours which account for < 1% of all solid ovarian tumors. It mostly occurs in 2–4° decades of life. Rapidly progessing symptoms/signs of androgen excess suggest the presence of and androgen secreting tumor.

Case

Two postmenopausal patients (50 and 57 year-old) were referred to our Endocrinology Clinic for evaluation of worsening hirsutism. Both of patients had manifestations of hyperandrogenism symptoms such as increasing growth and coarseness of hair body and androgenic alopesia. They had no voice and weight changes, fatigue or galactorrhea. Their modified Ferriman–Gallwey score were 19 and 36. Laboratory studies revealed normal complete blood counts, liver and kidney tests. Both of them have had diabetes (for 2 and 3 years, respectively). Endocrinologic work-up revealed normal hormone levels except for increased levels of total and free testosterone. Pelvic ultrasonographic findings were normal in first patient and there was 1 × 1 right ovarian mass in USG in second patient. Magnetic resonance (MR) imaging of the abdomen and pelvis were performed, which demonstrated normal adrenal glands in both. The left ovary measured 2.6 × 1.9 cm in first patient and no enlargement or ovarian tumor was visualised in second patient. Positron emission tomography revealed increased metabolic activity in left ovary of first patient and was normal in second patient. Bilateral salpingo-oophorectomy were performed in both patients. Pathologic examination revealed 1.5 cm mass in the left ovary in first patient and 2.5 cm mass in the right ovary of second patient which were both diagnosed as Leydig cell tumor. After operation virilisation signs have almost completely disappeared and testesterone levels returned to normal in two patients.

Result

Diagnosing Leydig cell tumors can be challenging, even it is seen in younger age groups, it should not be dismissed in postmenopausal hyperandrogenism differential diagnosis.

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P306

Adefovir diproxivil-induced acquired Fanconi's syndrome presenting as hypokalemia

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Introduction

Adefovir, commonly used for the treatment of hepatitis B, has dose-related nephrotoxicity, especially at doses of 60 mg daily and above. We describe a patient with Fanconi's syndrome after being on low dose adefovir for more than 5 years.

Case report

A 62-year-old Chinese man, a chronic carrier of Hepatitis B on adefovir 10 mg daily for over 5 years, presented with the incidental finding of profound yet asymptomatic hypokalemia (K 1.9 mmol/l, RI: 3.5–5.0 mmol/l). There were no

previous plasma electrolyte screens. He had weight loss, fatigue and bone pain for months. A high trans-tubular potassium gradient suggested renal potassium wasting. In addition, there was an associated non-anion gap metabolic acidosis, hypophosphatemia of 0.4 mmol/l (RI: 0.8–1.6 mmol/l) and hypouricemia of 154 μmol/l (RI: 250–550 μmol/l). The fractional excretion of phosphate was raised (59%, RI: 5–20%). The 24 h urine uric acid was 2778 μmol/day (RI: 500–5800 μmol/day) which was inappropriately normal. Urine protein excretion (24 h urinary total protein 0.880 g/day) and urinary amino acids were also raised, and glucose urinary dipstick was positive (2+). Dual energy X-ray absorptiometry showed osteoporosis. A diagnosis of acquired Fanconi's syndrome secondary to adefovir was made. Upon substitution with entecavir, we expect resolution of the electrolyte abnormalities to occur over the next few months.

Conclusion

It has been shown that adefovir depletes mitochondrial DNA which contributes mechanistically to proximal tubular dysfunction and hence acquired Fanconi's syndrome. Patients on adefovir therapy should therefore be screened periodically for electrolyte abnormalities, even when used at low doses.

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P307

Prolonged ventilator dependence in myxedema coma: a case report and review of the literature

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Introduction

Myxedema coma is a medical emergency and can be associated with respiratory failure. Intubated patients have been known to require persistent efforts in ventilator weaning, with one case reported to take 7 months before success.

Case report

A 77-year-old gentleman with known primary hypothyroidism presented with chest infection. He had defaulted thyroxine for the past 6 months, relying instead on complementary health products. Thyroid function test: free $T_4 < 2$ pmol/l (RI: 8–21), TSH > 100 mIU/l (RI: 0.34–5.60). He was administered both thyroxine and liothyronine promptly and covered with i.v. antibiotics but continued to deteriorate into myxedematous coma and required mechanical ventilation. Despite optimisation of thyroid medications and normalisation of thyroid function (fT_4 15 pmol/l), ventilator weaning was repeatedly unsuccessful for over 2 months. Electromyography and Nerve conduction test suggested critical illness polyneuropathy. Patient eventually made a competent decision (E4VTM6) for terminal extubation and passed on a day after extubation.

Conclusion

Our case report supports the observation in the literature regarding difficult ventilator weaning in myxedematous patients. Persistent respiratory failure may be due to a decreased respiratory drive, respiratory muscle myopathy and phrenic nerve neuropathy causing diaphragmatic dysfunction. Inefficient gas exchange from alveolar hypoventilation and concomitant pleural effusions may also contribute to respiratory failure. Underlying hypothyroidism is postulated to be an aggravating factor as well. In preventing hypothyroidism from progressing to myxedema coma, education to reinforce compliance is especially important in the elderly Asian population as they may remain wary of western medication and tend to rely on complementary health supplements. Clinicians should be cognisant that ventilator weaning in myxedematous patients may require prolonged efforts. Compliance to medications needs continuous reinforcement.

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P308

Graves' disease associated with severe hypoalbuminemi

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Introduction

The most common cause of thyotoxicosis is Graves' disease. Thyroid storm mostly presents with fever, tachycardia, arrthyhmia, jaundice, congestive heart failure and consciousness. We report a rare case of severe hypoalbuminemia with thyroid storm. To our knowledge it is the first case of severe hypoalbunemia due to thyrotoxicosis in literature.

Case

Forty one years old female had been followed up with the diagnosis of Graves' disease for 14 years. She was admitted with palpitations, shortness of breath, mild abdominal pain, nausea and complaints of discomfort. On examination, she had a temperature of 38.3 °C, arterial blood pressure 178/88 mmHg, irregular heart rate of 140 beats/min, respiratory rate of 22 breaths/min, mild pretibial edema and was seen nervous. Blood analysis revealed TSH of 0.005 IU/ml, FT_3 of 10.9 pg/ml, FT_4 of 3.5 pg/ml. ALT: 16 U/l, AST: 23 U/l, total-protein: 4.7 g/dl, albumin: 1.8 g/dl. Propylthiouracil, propranolol, Lugol's solution and dexamethasone were started. On the fourth day of treatment, TSH of 0.005 IU/ml, fT_3 of 2.97 pg/ml, fT_4 of 1.98 pg/ml. Total thyroidectomy was performed. Other underlying factors for hypoalbunemia were excluded. In the follow-up, total protein and albumin levels returned to normal limits.

Conclusion

Severe hypoalbuminemia, that can be seen in many serious diseases, may be encountered in the long term progress of thyrotoxicosis unexpectedly. Hypoalbuminemia should be evaluated during thyrotoxicosis.

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P309

Graves' disease associated with Alopecia areata developed after Hashimoto's thyroiditis

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Indroduction

Graves' and Hashimoto's thyroiditis are the most common autoimmune thyroid diseases. After Graves', hypothyroidism can develop spontaneously or as a result of medical treatment, radioactive iodine therapy or surgery. Development of Graves' after Hashimoto's thyroiditis is a rare condition. Alopecia areata (AA) is an autoimmune disease. It has association with other autoimmune diseases such as thyroid disorders, anemias and other skin disorders. We present a 41 years-old woman with Alopecia areata associated with Graves' disease who was previously diagnosed as Hashimoto disease.

Case report

Forty one year-old female patient admitted to emergency department with the complaint of excessive sweating, palpitation, discomfort, weight loss, trembling in hands, and diarrhea. She had been followed up with the diagnosis of Hashimoto disease for the last 6 years. TSH was < 0.005 IU/ml, fT_3: 17.01 pmol/l, fT_4: 4.35 pmol/l. L-thyroxine was stopped and propranolol was started. After 1 week, TSH was detected < 0.005 IU/ml, FT_3 was 14.34 pmol/l and FT_4 was 4.06 pmol/l. TSH receptor antibodies were > 50 U/l. Power Doppler of thyroid gland showed pattern of thyroid inferno (Figure 1). Propylthiouracil was added. Alopecia areata was observed during this period (Figure 2). After decline of fT_3 and fT_4 levels, Alopecia areata recovered.

Conclusion

The development of Graves' disease after Hashimoto disease is a rare condition. Alopecia areata can be associated with both Hashimoto's and Graves' diseases. It is the first case of Alopecia areata seen at the time of conversion of Hashimoto to Graves with increased TRAB levels. alopecia areata recovered completely after the decline of fT_3 and fT_4 levels with treatment.

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P310

Challenges in the management of hypogonadotropic hypogonadism

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Introduction

One of the main challenges in the management of male hypogonadotropic hypogonadism (HH) is in the restoration of fertility. We describe 2 patients with

HH and discuss the difficulties involved in their management.
Case (1)
A 23-year-old Chinese man presented with lack of secondary sexual characteristics. Examination revealed Tanner Stage 2 with descended testes measuring 6 ml bilaterally. Laboratory results: total testosterone (TT) <1 nmol/l, LH <1 IU/l, FSH <1 IU/l. Other anterior pituitary hormones and MRI pituitary were normal. As patient desired fertility soon, hCG 2000 units twice weekly was initiated with increase in TT levels to 15 nmol/l (RI: 5–30). Male sexual characteristics developed with enlargement of the testes to 15 ml bilaterally. However, semen analysis performed a year later showed persistence of azoospermia despite a normal TT level. As he could not afford human menopausal gonadotropin, hCG therapy was continued.
Case (2)
A 22-year-old Chinese man was referred for delayed puberty. Examination revealed micropenis and testes that were descended but measured 2 ml bilaterally. Laboratory results: TT: 2 nmol/l, LH 2 IU/l, FSH 3 IU/l. Other anterior pituitary hormones and MRI pituitary were normal. hCG was started but he failed to attain normal TT levels despite doses of up to 4000 units thrice weekly. Testosterone was subsequently started with the understanding that fertility would not be achievable. Secondary sexual characteristics eventually developed.
Learning point
Despite the presence of good prognostic features such as improvement in testicular size/normalisation of serum testosterone, azoospermia persisted in the 1st case after hCG treatment. However, it may take up to 24 months before a normal sperm count is attained post gonadotropin treatment. The cost of gonadotropins is a significant limiting factor in the management of HH.

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P311

Significance of HCG to distinguish parathyroid carcinoma from benign disease and in adding prognostic information: a hospital based study from Nepal

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Background
Parathyroid carcinoma is an rare endocrine malignant neoplasm resulting from the parenchymal cells of the parathyroid glands and infrequent cause of primary hyperparathyroidism whose diagnosis is very challenging. Therefore, our objective was to differentiate between benign and malignant hyperparathyroidism on the basis of excretion of HCG and its malignant isoforms in urine.
Materials and methods
It was a hospital based study carried out using data retrieved from the register maintained in Manipal Teaching Hospital from 1st January, 2008 and 31st August, 2012. The variables collected were urinary HCG, urinary HCG malignant Isoform, calcium, parathyroid hormone. All these biochemical parameters were analyzed in the Central Laboratory of our hospital by standard and validated methods.
Results
The urinary HCG 6.1 ± 6 fmol/mgCr was within normal range in benign hyperthyroidism. The urinary HCG was markedly high in three cases of malignant hyperparathyroidism. The maximum value of excretion in urine for HCG was 2323 fmol/mgCr. The excretion of malignant isoform of HCG in urine was 0 in benign hyperparathyroidsm and in four cases of malignant hyperparathyroidism which falls in category of persistantly low HCG. The maximum excretion of malignant isoform of HCG in urine was 1.8 which falls in category of markedly high HCG.
Conclusion
The elevated expression of standard or hyperglycosylated HCG is an adverse prognostic indicator and helps to differentiate between benign and malignant hyperparathyroidism.

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P312

Report of a case of hyperandrogenism in a postmenopausal woman

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Objective
To report a case of hyperandrogenism attributable to the presence of an ovarian Leydig cell tumor secreting testosterone in a postmenopausal woman.
Methods
The laboratory, radiologic, and pathologic findings in our case are described.
Results
A 59-year-old woman presented with a history of gradual increase in facial and body hair, scalp hair loss, male pattern baldness, and deepening of her voice, beginning 5–6 years before. She had two normal pregnancies and spontaneous menopause at age 47 years. She had hypertension and hyperlipemia. Laboratory tests showed elevated levels of total testosterone (575 ng/dl), very low gonadotrophin levels and normal levels of 17-OH-Pg, Androstendione and DHEA-S. Her high level of testosterone was not suppressed with dexamethasone. Ultrasound study and abdominal computed tomographic scan showed the adrenal glands to be normal in size. Transvaginal ultrasonography revealed no tumor. Bilateral oophorectomy was performed, and an ovarian Leydig cell tumor in left ovary was diagnosed, in the contralateral ovary a stromal hyperplasia was found (non-neoplastic condition of the ovary also associated with clinical manifestations of hyperandrogenism from ovarian production of male hormones). The postoperative serum testosterone level returned to normal and the patient showed a slow regression of clinical symptoms after the surgical intervention.
Conclusion
Our case illustrates that a virilizing ovarian tumor can be small and elude imaging studies, but may be detected by means of well-considered clinical management. And, particularly in our case, we found a Leydig cell tumor and a contralateral stromal hyperplasia.

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P313

Primary octreotide therapy in a patient with pituitary adenoma cosecreting GH and TSH

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Pituitary adenomas which co-secrete GH and TSH accounts for $<0.2\%$ of all adenomas of the pituitary. Symptoms of hypertyroidism are usually masked by clinical manifestations of acromegaly. Here, we have reported the result of the primary one year long medical therapy in a case who was diagnosed to have adenoma co-secreting GH and TSH.
Case
Seventy-years-old female patient has admitted with the complaints of excessive sweating, enlargement of hands and feet. In physical examination there were frontal bossing, coarsening of facial structures and acral enlargement. In hormone tests, GH level was: 17 ng/ml and IGF1 level was: 607 ng/ml (64–188 ng/ml). GH levels were not suppressed during glucose suppression test. Her serum TSH, fT_4 and fT_3 levels were elevated. Serum alpha subunit was also high (80 IU/l). In thyroid ultrasonography, we have detected goiter. In thyroid scintigraphy, homogenous diffuse hyperplasia of both lobes was reported. In pituitary MRI, a 13×16 mm sized macroadenoma on the left handside of the pitiutary gland. The diagnosis of acromegaly was made with high suspicion of TSH co-secretion from the adenoma. Since the patient had dilated cardiomyopathy and obstructive sleep apnea syndrome, the operation could not be done. Octreotide LAR was started in a dosage of 30 mg/28 days. The patient was evaluated at the first year of the therapy. IGF1 level decreased (285 ng/ml) and the size of the adenoma was reduced (11×10 mm). Serum fT_4, fT_3 and TSH levels were normal together with normalization of serum alpha subunit.
Conclusion
Coexistence of GH secreting adenoma and TSHoma is rare. In such cases, transsphenoidal surgery must be the first choice for therapy. However when operation is contraindicated as it occurred in our case or when the lesion cannot be excised completely resulting in residual mass, somatostatin analogues should be preferred as an alternative therapy.

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P314

Follicular thyroid cancer with functioning lung metastasis

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Background
Functioning metastasis from a primary thyroid cancer are exceedingly rare. Failure to proceed to hypothyroidism after total thyroidectomy denotes remaining hormone production from functioning metastasis. Radioactive iodine ablation will ablate these remaining tissues. We present the case of a patient with follicular thyroid cancer, lung metastasis and detectable thyroid hormones after thyroidectomy.
Clinical presentation
The patient is a 71-year-old Filipina who had recurrent thyroid nodules for the past 22 years. She underwent four surgeries and the most recent was completion thyroidectomy for a $6.7\times6.4\times5$ cm lobulated mass in the left thyroid bed. Preoperative TSH suppressed at 0.008 mIU/l (NV: 0.3–3.8) and free thyroxine was elevated at 36.2 pmol/l (NV: 11–24). Thyroid scintigraphy using 6 mCi of technetium 99m pertechnate showed functioning thyroid tissues with a conglomerate size of 8×5.6 cm. Chest CT scan revealed numerous small well-defined nodules scattered in both lungs. She was treated with Methimazole 20 mg daily for 4 weeks prior to surgery. Histopathology showed follicular carcinoma in the left thyroid lobe, the strap muscles and cervical lymph nodes. Three months after thyroidectomy, her TSH remained suppressed at 0.2 mIU/l and FT_4 detectable at 11.7 pmol/l. Diagnostic whole body scan using 3 mCi Iodine-131 showed two small foci of functioning thyroid remnant measuring 1.6×1.0 cm and 0.6×0.6 cm. Both lungs showed increased tracer uptake. A diagnosis of functioning lung metastasis was made. The patient underwent radioactive iodine ablation with 150 mCi of Iodine-131. Thyroid function tests done one month later revealed an elevated TSH 33.4 mIU/l and low FT_4 at 8.2 pmol/l indicative of successful ablation of functioning thyroid tissues.

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P315

Wegener's granulomatosis in a patient with vitamin D deficiency

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Wegener's granulomatosis is characterized by necrotizing granulomatous vasculitis. It occurs initially in a localized form, disseminates in various degrees and particularly involves the respiratory tract and kidneys. It is an ANCA-associated vasculitis, a systemic disease of autoimmune aetiology. Recently vitamin D deficiency has been associated with the development of autoimmunity. The aim was to present a case of Wegener's granulomatosis in a patient with vitamin D deficiency induced by gastric surgery for the treatment of morbid obesity.
A patient, female aged 47 years, presented with chronic episcleritis, conjunctivitis, retroorbital pain and erythema of the left eye over the course of 2 years. The patient had gastric surgery for the treatment of morbid obesity and had also been subjected to thyroidectomy for the treatment of a thyroid nodule. On clinical examination she had a hemorrhagic rash over the lower extremities and bilateral hearing loss. Laboratory investigations revealed vitamin D deficiency, $25(OH)D_3$ levels being 11.7 ng/ml (normal levels >30 ng/ml), microscopic hematuria, proteinuria, and positive c-ANCA. Imaging studies revealed the presence of nodules in the lungs and signs of left orbital inflammation. The diagnosis of Wegener's granulomatosis was made. I.v. methylprednisolone pulse therapy was initiated and vitamin D was administered orally with subsequent sustained improvement.
In conclusion, the case of a patient with Wegener's granulomatosis and vitamin D deficiency after gastric surgery for morbid obesity is presented. Vitamin D deficiency is known to be associated with the development of systemic autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. Vitamin D deficiency induced by gastric surgery may be implicated in the pathogenesis of a systemic autoimmune disease with ocular manifestations in this patient.

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P316

Mixed medullary-follicular carsinoma of the thyroid: two case reports

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Mixed medullary-follicular carcinomas (MMFC) of the thyroid are rare tumors which represents $<0.15\%$ of all thyroid tumors showing the morphological and immunochemical properties of both parafollicular and follicular cell lineages. Cases were immunoreactive for both calcitonin and thyroglobulin.
Case 1
A 25-year-old female patient referred us for evaluation of a thyroid mass located on the left lobe. Ultrasonography (US) revealed 38 mm solid isoechoic nodule with well-defined margins on the left lobe. An US-guided fine needle aspiration (FNA) was performed and reported as follicular neoplasm. A total thyroidectomy with central lymph node dissection was performed. Immunostaining revealed that the tumor cells were diffusely positive for calcitonin and chromogranin. Further, there were scattered follicles that stained positively with antibody against thyroglobulin. The histological findings in correlation with the immuneprofile, support a diagnosis of MMFC. Post-operatively calcitonin level was 2.69 pg/ml. Radioactive iodine ablation with 150 mCi and L-thyroxine supression theraphy had given to the patient.
Case 2
A 46-year-old female patient admitted with enlargement of a neck mass. US revealed 33 mm solid isoechoic nodule with ill-defined margins and micro-calcifications on the left lobe of thyroid. US-guided FNA was reported as thyroid neoplasm with differentiation or anaplastic transformation. Patient's serum level of calcitonin was 1802 pg/ml (normal <12 pg/ml). Results of blood testing for pheochromocytoma and hyperparathyroidism were within the normal range. Total thyroidectomy with central lymph node dissection was performed. The histological findings were consistent with MMFC with metastatic lymph nodes. The serum calcitonin level dropped to 3.75 pg/ml following the surgery.
Conclusion
The majority of MMFC occurs in a sporadic form, rarely it can be a component of MEN type 2. MMFC, medullary and follicular thyroid cancer behave differently in terms of clinical course and management. Identification of MMFC by FNA may be difficult, the proper immunostaining panel could have showed the different aspects of the mixed tumor.

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P317

Cyclosporine induced autoimmune thyroid disease: presentation of two cases

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Immune supressive patients due to any cause (disease, medication, etc.) rarely have autoimmune diseases. In this presentation, two patients diagnosed with Graves' disease and subacute thyroiditis while taking cyclosporine therapy will be discussed.
Case 1
A 47-year-old female patient applied with weight loss, sweating and tremor. She had been on cyclosporine therapy for 3 years because of hypoplastic anemia. In laboratory tests; TSH: 0.005 uIU/ml (0.27–4.2), fT_4: 7.77 ng/dl (0.9–1.7), and fT_3: 28.56 pg/ml (1.8–4.6). Anti-TPO, Anti-TG and TSH receptor antibodies were positive. Ultrasound revealed enlarged thyroid gland with diffuse parenchymal heterogeneity. Thyroid scan showed increased uptake, which was diffuse and homogenous in pattern. On radioiodine uptake test: 4th hour uptake was 46% (15–25%) and 24th hour uptake was 27% (25–35%). The patient was diagnosed as Graves' disease. With thyromazol therapy euthyroid state was achieved.
Case 2
A 41-year-old female patient admitted with pain and tenderness in the thyroid area. She had been using cyclosporine during the last 2 year period for psoriases. In laboratory tests; TSH: 0.015 uIU/ml, fT_4: 1.48 ng/dl, and fT_3: 3.92 pg/ml. Anti-TPO and TSH antibodies were negative while anti-TG antibodies were positive. Ultrasonography showed multiple hypoechoic thyroid nodules with ill-defined margins on the basis of chronic thyroiditis. In the thyroid scan there were

supressed areas in nodular pattern and a global nonhomogenous uptake. There was low radioiodine uptake in the radioiodine uptake test. The patient was diagnosed as subacute thyroiditis. Nonsteroidal anti inflamatory drug and beta bloker were given. One month later, her thyroid function tests were within normal limits.
Conclusion
Cyclosporine rarely can be a cause of autoimmune disease by an unknown mechanism of abnormal modulation of the immune system. Patients taking cyclosporine especially those having another autoimmune disease or family history of thyroid disease must be carefully monitorized with thyroid function tests.

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P318

Never too late to discover some extra thyroid tissue

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Introduction
Ectopic thyroid is a rare entity, resulting from developmental defects at early stages of thyroid embriogenesis. It's prevalence is 1/100 000–300 000 in general population and 1/4000–8000 in patients with thyroid disease. This condition is more common in females, in Asians and may occur at any age, although it's most common at younger ages. The most frequent location of ectopic thyroid tissue is at the base of the tongue. In 70–75% of cases is the only thyroid tissue present. The mean age of presentation is at 40.5 years. The most common symptoms are related to the growth of lingual thyroid (dysphagia, disphonia, sleep apnea and in more severe cases respiratory obstruction and hemorrhage) and hypothyroidism, especially in the absence of orthotopic thyroid.
Case report
We present a case from a Caucasian white male, 82 years, assymptomatic, with subclinical hypothyroidism diagnosed at routine clinical evaluation. The Ultrasound scan shwowed a decreased size thyroid gland and head and neck CT, ordered because of other clinical problems showed a 25 mm sublingual thyroid gland, confirmed by Scintigraphy. He also did a Laringoscopy wich confirmed a well defined sublingual mass. He was medicated with L-thyroxine and as he kept asymptomatic no other treatment was needed.
Conclusion
This case shows a case of a caucasian male patient with a rare congenital condition, more common in Asian women. He had a lingual ectopic gland and also a orthotopic thyroid, which only happens in 25–30% of these cases. This is probably the main reason why he kept assymptomatic most of his life, until the age of 82 (mean age of diagnosis 40.5 years) and his clinical presentation consisted only in a subclinical hypothyroidism. So, in clinical practice we should be carefull precluding any condition just based on probability.

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Everolimus. a new therapeutic target in the metastatic neuroendocrine carcinoma

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Introduction
Everolimus is a drug selective inhibitor of mTOR and approved by the FDA in 2011 for treatment of neuroendocrine tumors of pancreatic origin unresectable. Our objective was to evaluate the efficacy and safety of everolimus in a patient with malignant pancreatic head insulinoma.
Case
This is a 31 year old man who complains of epigastric pain, dizziness, sweating and syncope related to exercise. During admission is objectively severe hypoglycemia (<20 mg/dl) requiring steroids and diazoxide to maintain normoglycemia. In the analyzes presented insulinemias inappropriately high (30.4–36.5 mU/ml), increased catecholamines, 5HIAA and hypertransaminemia. A abdomen magentic resonance show a lobulated injury in pancreatic head of $5\times 4\times 6$ cm and multiple metastases in liver. In a laparotomy, we observed multiple interaortocavas gastroepiploic adenopathies, so we rejected the pancreatectomy. Histopathological examination revealed infiltration by neuroendocrine carcinoma type malignant insulinoma G2 (2 mit/10cga and Ki-67 $>7\%$). A Ostreoscan was negative. Chemotherapy was discarded for the low mitotic index, also the somatostatin analogs for the negativity octrescan. Finally, we decided to treat the patient with two liver chemoembolization, followed by treatment with everolimus 10 mg/24 h.
Results
During follow-up of 2 year, the tumor masses have remained similar size and radiological appearance, without evidence of new injuries. Hypertransaminemia remained stable. The patient remained euglycemic without specific treatment, even dietetic, and performs a normal life. Tolerance drug has been good without important adverse reactions.
Conclusions
Everolimus is a new treatment for pancreatic neuroendocrine tumors, especially useful in patients with great local extension or metastasis, when the surgery is not curative. Is a targeted therapy with antiproliferative effect, what slows the hormonal release, resulting in our case a control of carbohydrate metabolism. It is therefore a drug to be considered in these tumors, because good results can be obtained, as is the case in our patient.

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P320

Torsade de pointes caused by gluten sensitive enteropathy leading to multiplex endocrine failure: case report

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A 55 year-old man was brought to the emergency room because of hypotension, fatigue, fever and pain in the left shoulder. The blood glucose, sodium, potassium levels were normal. ECG showed 65/min sinus rhythm with negative T waves in the precordial leads. Blood pressure was 70/50 mmHg. Suddenly torsade des pointes occurred, which was converted to sinus rhythm with 300 mg amiodarone. Coronarography showed no significant stenosis on epicardial coronaries. During the intervention supraventricular tacyhcardia occurred for 30 s, and disappeared after 150 mg amiodarone, ECGs showed no PQ prolongation, 76/min sinus rhythm and diffuse T-wave depression. Echocardiography found EF 38%, anterior, inferior, septal akinesis. On the 4th and 7th day of observation 35/min bradycardia occurred with junctional and ventricular extrasystole requiring defibrillation with 200 J. TSH measurement suggested hypothyroidism (TSH: 13.89 mIU/l), severe hypocalcaemia suggested hypoparathyroidism. Serum total calcium level was: 1.82 mmol/l, PTH: 1.5 pmol/l. Low serum hydrocortisone value revealed adrenal insufficiency. As the suggested diagnosis was polyglandular autoimmune syndrome we performed autoimmune screening and found anti transglutaminase antibodies. However, further autoimmune screening showed no sign for other autoimmune disesases. Gluten-free diet, hydrocortisone, L-thyroxine, calcium, vitamin D3, and testosterone supplementation started, ICD was implanted. Torsade never occurred again. Last results showed normal Ca, TSH, fT_3, fT_4 values, mildly lower testosterone, suppressed ACTH and mildly elevated cortisol levels.
Conclusion
We found gluten-enteropathy caused persistent polyglandular endocrine failure leading to torsade de pointes tachycardia requiring several reanimation and ICD implantation without having autoimmune origin.

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P321

Atypical parathyroid adenoma presenting with severe hypercalcemia: a case report

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Introduction
Atypical parathyroid adenoma (APA) is a difficultly diagnosed tumor, including some histological features of parathyroid carcinoma (PC). In literature, no definite criteria are considered to be present to distinguish preoperatively APA from PC. It is difficult to distinguish APA and PC intraoperatively. Our report, a case applying with high levels of calcium (Ca) and intact parathyroid hormone (iPTH) and diagnosed with APA was presented.
Case
A 65-year-old woman applied to our clinic with arthralgia, myalgia and fatigue. She had severe hypercalcemia (serum Ca 17.2 mg/dl), hypophosphatemia (serum phosphate 1.9 mg/dl) in conjunction with an elevated iPTH level of 879 pg/ml. There was not a lesion consistent with parathyroid adenoma in neck ultrasonography. Tc-99m sestamibi scintigraphy and neck magnetic resonance imagination scans revealed a suspicious lesion that can be a parathyroid adenoma at the posterior region of left thyroid lobe. I.v. hydration and diuretic treatment were given to the patient. Six sessions of hemodialysis were performed. Parathyroidectomy guided by intraoperative gamma probe was performed, and a $3.5\times3\times2$ cm sized parathyroid adenoma was excised. Histopathologically, well-circumscribed parathyroid cell proliferation was seen with solid growth pattern. Round to oval shaped proliferative cells were surrounded by thick fibrous capsule and seperated by dense fibrotic bands. No marked pleomorphism and mitotic figure was present. Ki-67 proliferation index was $<1\%$, and no vessel invasion, complete capsule invasion and invasion to the adjacent structures were observed. In an area, parathyroid cell proliferation became invaded into the capsule, no complete invasion of the capsule was observed. Findings were considered to be related to APA.
Conclusion
No strict clinical and histological criteria are present to distinguish PC from APA. The existence of local recurrence or metastatic disorder is the single and reliable characteristic in distinguishing between benign and malignant parathyroid disorders.

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P322

Severe hyponatraemia in patients admitted to acute medical unit

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In a retrospective cohort study, we reviewed the medical notes of patients with severe hyponatreaemia admitted to acute medical unit at Blackpool Hospital between January and March 2012. We assessed the severity, causes of hyponatraemia, length of stay and mortality in patients with hyponatraemia.
We identified 25 patients with plasma sodium $\leq$120 mmol/l, 5 of whom were admitted twice during the study period. Their average age was 70 (range 30–91) years, 16 were female and 9 were males. Three of them with sodium $<$ 110 mmol/l and the others had sodium 110–120 mmol/l. Twelve patients (48%) had acute hyponatraemia, eight patients (32) had subacute hyponatraemia of <6 months duration, and five patients (20%) had chronic hyponatraemia for more than 6 months duration.
Reasons for admission include confusion in 24% of patients, collapse in 20%, falls in 20%, and vomiting 8%.
Elven patients (44%) were found to have hypovolaemic hyponatraemia, nine patient had hypervolaemic hyponatraemia related to heart and liver diseases, and five patients had normovolaemic hyponatraemia related to SIADH related to drug therapy.
Hyponatraemia was corrected within a week in eight patients (32%), and was corrected between 1 and 4 weeks in seven patients (28%), and never corrected in seven patients.
Length of hospital stay was less than a week in 13 patients (56%), and 44% of patients stayed for more than a week. Mortality was 16%.
Severe hyponatraemia in acute medical admission is associated with prolonged length of hospital stay, and high mortality.

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P323

Androgen abuse complicated by polycythemia in a man with acquired immunodeficiency syndrome (AIDS)

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Introduction
Male hypogonadism secondary to AIDS is common and may present with non specific symptoms. Patients may self-diagnose hypogonadism and seek unauthorised androgen therapy. We report a male patient who developed polycythemia after receiving unauthorised sources of androgen therapy.
Case report
A 42 years old man with AIDS diagnosed 10 years ago has repeatedly refused antiretroviral therapy. His most recent CD4 count was 314 cells/μl. His hemoglobin and hematocrit were elevated at 18.3 g/dl (RI: 13–17) and 52% (RI: 41–51) respectively at a recent review. He complained of lethargy and decrease in libido of 6 months duration. There was no headache or other symptoms of hyperviscosity. He is a non-smoker and there was no known pre-existing cardiopulmonary disease. He was well hydrated and there was no hepatosplenomegaly on examination. Total testosterone was noted to be elevated at 34 nmol/l (RI: 5–30 nmol/l) and FSH and LH suppressed at <1 IU/l. He admitted to taking complementary health products to boost his virility as he had presumed that he has hypogonadism. Advice was given to stop the health products which are likely to contain testosterone. Repeated blood tests done 2 months later showed normalisation of the hemoglobin, hematocrit as well as the FSH, LH and testosterone levels without the need for testosterone therapy.
Discussion
Male hypogonadism secondary to AIDS can result from testicular infiltration by opportunistic infections, medications suppressing the hypothalamic–pituitary–gonadal axis or be associated with advanced immunosuppression. It is important to routinely screen for symptoms of hypogonadism and if the diagnosis confirmed biochemically, to start testosterone replacement therapy with close monitoring of the hematocrit. Clinicians must be cognisant of possible androgen abuse. Self initiation of androgen therapy with no monitoring can result in polycythemia and dire consequences.

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Abstract withdrawn.

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P325

A rare presentation of DRESS-associated thyrotoxicosis and myocarditis: case report

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Introduction
Drug rash with eosinophilia and systemic symptoms (DRESS) is a severe drug eruption with systemic involvement. Visceral manifestations consisting of fulminant hepatic failure, myocarditis, interstitial nephritis, pneumonitis and autoimmune hypothyroidism are known sequela. We report a case of DRESS syndrome post Bactrim (sulfamethoxazole and trimethoprim) therapy complicated by both myocarditis and autoimmune thyrotoxicosis.
Case report
A previously well 18 years old gentleman was prescribed Bactrim for acne vulgaris and developed DRESS syndrome 2 weeks later, He was started on high dose steroids. 2 months into steroid therapy, he presented at the Emergency Department for fever and decreased effort tolerance. He was critically ill with tachycardia (Heart Rate 150 bpm), hypotension (Blood Pressure 70/40 mmHg)

and an ejection fraction of 20% on 2D-echocardiography.There were multiple episodes of unstable ventricular tachyarrhythmias requiring cardioversion and intubation. Thyroid panel revealed free T_4 49 (RI 8–21 pmol/l), TSH 0.04 (RI 0.34–5.60 mIU/l) and free T_3 6.4 (RI 3.5–6.0). Thyroid autoimmune markers were positive (TPO Ab 68.8 (RI 0–60 IU/ml), TRAb 14.8 (RI 0–1.5 IU/l). He was treated for possible thyroid storm with propylthiouracil and hydrocortisone. He also had severe myocarditis with hypotension refractory to inotropic support and intra-arterial balloon pump. Extracorporeal membrane oxygenation (ECMO) was initiated but patient continued to deteriorate and unfortunately passed away 5 days after admission.

Conclusion

Despite high dose immunosuppression and cessation of the causative agent post DRESS, multiple autoimmune sequelae may develop and may unfortunately be life threatening. Both patients and clinicians need to be cognisant of this. Early diagnosis and treatment may potentially be life saving.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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Radiation-induced primary hypothyroidism in patients with head and neck cancer

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Background

Hypothyroidism is a frequent side effect of external radiotherapy of the cervical region for head and neck cancer. Up to 33% of irradiated patients developed primary hypothyroidism within 2 years after radiation therapy. The risk of hypothyroidism increases with radiation dose.

Patients and methods

Four patients (2M/2F), aged 53.3 ± 13.9 years, resident both in iodine sufficient ($n=2$) and iodine deficient areas ($n=2$), were diagnosed with hypothyroidism after cervical irradiation for head and neck cancer. TSH and FT_4 were measured by chemiluminescence.

Case reports

Patients received external-beam radiation therapy delivered in the form of photon beams, for undifferentiated cavum carcinoma, oropharyngeal cancer, epidermoid spinocellular carcinoma of palatine tonsil and laryngeal carcinoma; median radiation dose was 63 Gy. Median time from radiotherapy to diagnosis of hypothyroidism was 3.5 years (range: 0.5–9 years). Median TSH at diagnosis was 55 mIU/l (range: 5.97–100 mIU/l). TPO antibodies and antithyroglobulin antibodies were negative in all patients. All patients but one presented marked thyroid atrophy. Hypothyroidism was complicated with pericarditis in two patients.

Conclusion

Thyroid screening is mandatory for an early diagnosis of radiation-induced primary hypothyroidism and for prevention of hypothyroidism complications.

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P327

A case of type 1 diabetes represented by diabetic ketoacidosis after isotretinoin therapy: is it a result or coincidence?

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Well-known environmental factors which can lead to type 1 diabetes are viral infections, vaccines, diet, exposure to cow milk in infancy, vitamin D deficiency, drugs, maternal age, preeclampsia and low birth weight. Isotretinoin is an effective drug for acne treatment. It can cause dyslipidemia, elevated liver enzymes, insulin resistance and type 2 diabetes. Our case is type 1 diabetes developed after the use of isotretinoin and represented with diabetic ketoacidosis.

Case

18-years-old male patient has admitted to hospital with complaints of abdominal pain, increased sense of thirst, frequent urination and dry mouth. In physical examination, he was dehydrated with hypotension and dry oral mucosa. He did not have family history of diabetes. With further questioning, we have learnt that he was using isotretinoin for acne treatment for 7 months. In laboratory examination, his plasma glucose level was 400 mg/dl, had heavy ketonuria and acidosis in arterial blood gas. He was hospitalized in endocrinology ward with diagnosis of diabetic ketoacidosis. Hydration with isotonic saline together with insulin infusion, potassium replacement and dextrose infusion were given to the patient. His anti GAD was positive and C-peptide level was very low leading to diagnosis of type 1 diabetes. After amelioration of ketoacidosis, we have applied intensive insulin therapy and blood glucose levels were regulated.

Conclusion

Isotretinoin is related with metabolic syndrome and type 2 diabetes because of worsened insulin resistance. However, there are few cases of type 1 diabetes in literature. Anti GAD positivity is suggestive of underlying autoimmune mechanisms triggered by the drug. Patients should be monitored with frequent plasma glucose levels during treatment.

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Lithium-associated hyperparathyroidism: a case report

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Introduction

Lithium carbonate therapy has continued to be a mainstay treatment for bipolar disease and schizoaffective disorders. Hypercalcemia and hyperparathyroidism (HPT) is an underappreciated, but relatively common occurence, with a prevalence ranging from 6.3 to 50% in patients requiring long-term lithium therapy. It is stil unclear whether lithium initiates HPT or promotes an underlying subclinical state. Lithium antagonizes the calcium-sensing receptor (CASR) resulting in an increase in the threshold level of calcium required for suppression of serum PTH. This is validated by the fact that many patients with lithium associated hyperparathyroidism (LAH) have inappropriately low-normal urinary calcium excretion. LAH reflects a spectrum of disease from single to multigland involvement with a variable responce of each parathyroid gland to the continous PTH stimulation.

Case report

A 52-year-old female with bipolar disorder who had been treated with 900 mg lithium per day for 2 years, was referred to our endocrine out-patient clinic for evaluation of hypercalcemia. She denied having any symptoms related to hypercalcemia. Her blood and urine tests were as follows: serum calcium: 10.9 mg/dl (normal range: 8.6–10.2 mg/dl), phosphorus: 3.4 mg/dl (normal range: 2.6–4.5), creatinine: 0.8 mg/dl (normal range: 0.95), PTH: 191 pg/ml (normal range: 15–65), 24-h urinary calcium: 66 mg/day (normal range: 100–321). Her bone mineral densitometry showed reduced *T*-score: -2 (neck of femur), -1, 3(spine). Parathyroid scintigraphy with Tc-99m MIBI showed a parathyroid pathology with an isotope retention at lower pole of left thyroid gland. She was diagnosed as LAH. After consultation with her psychiatrist, lithium therapy was stopped. As she did not meet the criteria for parathyroid surgery of asymptomatic hyperparathyroidism, a conservative approach to therapy was thought to be appropriate. After 2 weeks of cessation of lithium therapy, her serum calcium decreased to 10.1 mg/dl.

Conclusion

Approximately 10–15% of lithium treated patients become hypercalcemic with findings suggestive of HPT. By routinely monitoring serum calcium levels and long-term follow-up, healthcare providers can reduce the morbidity and improve the quality of life.

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Thyrotoxic periodic paralysis in young Caucasian

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Thyrotoxic periodic paralysis (TPP) is uncommon complication of Graves' disease mainly observed in young males of Asian origin. It is rarely seen among

white hyperthyroid patients (0.1–0.2%), usually presented as lower extremities muscle weakness of sudden onset, probably cause by hypokalemia. Possible mechanism of hypokalemia is an increased sodium–potassium–adenosine triphosphatase pump activity with consequent massive shift og potassium from extracellular to the intracellular compartment, with no change in the total body potassium levels.
We presented a 33-year-old Caucasian male with significantly increased levels of free thyroxine associated with newly diagnosed diffuse toxic goitre. Unexpectedly, patient experienced sudden muscle weakness with immobility at the second hospital day. Because of suspicion that this condition was an adverse effect of propylthiouracil (PTU), drug was immediately stopped. Repeated biochemistry findings revealed extremely low levels of serum potassium and elevated creatine phosphokinase levels (CPK). With careful potassium substitution via parenteral and oral route as well by management with nonselective beta blocker propranolol, gradual restoration of muscle strength and full mobility was achieved within few hours. Control biochemistry showed normal potassium levels. Further thyrosuppresive treatment was continued with methimazole and propranolol until euthyroid state was achieved. Then the patient underwent total thyroidectomy.
Despite the fact that is rare condition, clinicians must bear on mind the possibility of TPP presence mostly registered in the males and on the beginning of Graves' disease management.

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P330

Persimonious indications for thyroidectomy in chronic thyroiditis

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Background
Chronic inflammatory processes of the thyroid represents an important proportion of the gland's pathology but the majority of them must be treated by nonsurgical methods, thyroidectomy remaining circumstantially indicated.
Patients and methods
In 14 cases (3%) from 464 operations for different thyroid lesions lesions we have encountered authentic inflammatory processes in nine cases of Hashimoto's thyroiditis (two associated with papillary thyroid carcinoma and one with malignant lymphoma), two cases of Riedel's thyroiditis and de Quervain's thyroiditis , tuberculous thyroiditis and thyroid actinomycosis one case each respectively. The clinical and imaging data. biological evaluation and titer of the antibodies but the intraoperative estimations together with the paraffine examination were determinative for the diagnosis.
Results and discussions
Firm diagnosis of thyroiditis was rarely affirmed before the operation, indication for surgery being formulated on clinical criterions dominated by the cancer suspicion. Among them the diffuse or (multi)nodular thyromegaly with a dominant nodule with recent apparition and accelerate growing, hard consistence, compressive or celsian features and adenopathy in temporal or geographic proximity of the Cemobyl disaster.
More added the imaging tests but especially the suspect aspects of the FNAB (follicular smears or with Hurthle cells) and also of the frozen sections. Certainty diagnosis was established by the paraffin examination not always without hesitations or re-examinations (In one case of Hashimoto's thyroiditis the final diagnosis was a malignant lymphoma). A large near total removal of the thyroid gland decided after Intraoperative findings induced for the most of our patients an obvious tendency toward hypothyroidism which must be monitorised and corrected for prolonged periods.
Conclusions
All the diagnosis resources must be exhausted for the precise diagnosis of the inflammatory lesions of the thyroid to avoid the unnecessary surgery. On the other side the overstimulation by the TSH of the thyroid tissue affected by the inflammatory process represent an important neoplasia producing stimulus.

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P331

The coexistence of vaginismus with arthritis after pharmacological treatment of hyperthyroidism: is it only a coincidence?

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Introduction
Both thyroid dysfunction and sexual dysfunction in young women are relatively common. Even is described the correlations between various types of sexual dysfunction in women, and the severity of hyperthyroidism. However, it is known to us a description of the coexistence of vaginismus with arthritis, a relatively rare complication of antithyroid treatment.
Case report
25-year-old woman was admitted to hospital with symptoms of polyarthritis which occurred during treatment of hyperthyroidism propylthiuracyl. Methimazol previously used, was discontinued due to skin allergy. Clinical and biochemical signs of hyperthyroidism were relatively mild (TSH = 0.13 mU/l, FT_3 4.8 pg/dl, FT_4 1.65 ng/dl) with slightly elevated antithyroid autoantibody. The ultrasound structure of thyroid was heterogeneous. However, polyarthritis symptoms were quite severe, with the presence of rheumatoid factor. The patient also had complained symptoms of vaginismus, which confirmed her husband. Propylthiouracyl withdrawal was not enough for the resolution of symptoms. After administration of 40 mg of prednisone and 5 mg bisoprolol, joint symptoms subsided and we observed clinical euthyreosis. Further treatment follow in the country of residence of our patients. Thyroidectomy was performed successfully, and the patient's condition, according to her family remained good. Unfortunately, we know nothing about the further course of vaginismus.
Discussion
Short treatment time did not allow us to more detailed diagnosis of the causes and possible treatment of vaginismus. It is difficult to clearly identify the possible pathological mechanism linking disorders seen in our patient. Because of mild hyperthyroidism, hormonal disturbance are unlikely as a cause of vaginismus. More likely, it seems already described in Sjogren's syndrome, is autoimmune mechanism However, this remains speculation.

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Developmental Endocrinology

P332

Crystal structure of the complex between an insulin-like peptide (DILP5) and an ILP binding protein (IMP-L2) from *Drosophila melanogaster*

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The insulin signalling system including the insulin receptor tyrosine kinase (IRTK) is evolutionarily ancient and appears in the first multicellular organisms (Cnidarians). The *Drosophila melanogaster* genome contains seven genes for insulin-like peptides (ILPs) that are expressed in neurosecretory cells in a highly tissue- and stage-specific pattern, DILP1-7. There is however only one IRTK (dIR). This system is important in the regulation of metabolism, growth, reproduction and lifespan.
We reported in 2011 the crystal structure of Drosophila insulin-like peptide 5 (DILP5) (expressed in *Saccharomyces cerevisiae*) at 1.85Å resolution, as well as its biological and receptor binding properties. DILP5 shares the canonical fold of the insulin peptide family and form dimers that differ from the mammalian and hagfish insulin dimers.
Drosophila melanogaster also has a circulating ILP binding protein, called imaginal morphogenesis protein-Late 2 (IMP-L2), with no equivalent in mammals. It was cloned, expressed and purified in 2000 and was shown to bind human insulin and insulin-like growth factors. We showed in 2011 that it also binds DILP5. The knockout of IMP-L2 is embryonic lethal.
We now report the solution of the crystal structure of IMP-L2 in the free form as well as bound to DILP5. Recombinant IMP-L2 was expressed in a Baculovirus expression system, purified and crystallized with selenium as heavy atom.
IMP-L2 showed as predicted from the sequence a bilobed structure with two IgG beta sheet modules (3 strands for IgGI and 4 for IgGII), folded together into a 'baseball glove'. The structure is very different from the known partial structure of IGFBP5 which is mostly alpha-helical.

The complex of IMPL-2 with DILP5 showed a multimeric structure with two DILP5 molecules bound into the grooves between the beta sheets of two distinct IMP-L2s in a symmetrical tetrameric IMPL-2. The structural differences between the free and bound forms will be discussed.

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P333

Changes in sexual orientation in gender identity disorder: evaluation of their association to sex reassignment surgery and cross-sex hormone treatment

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Objective
Sexual orientation in males and females is considered to be determined in early life and to be virtually unchangeable in the course of adulthood. In contrast some transsexuals report a change in their sexual orientation most overt following sex reassignment procedures. The reason for this phenomenon is unknown.
Methods
We asked 106 transsexual (66 MtF and 40 FtM) patients from our endocrine outpatient clinic to complete a questionnaire, retrospectively evaluating the history of their gender transition phase. A special focus was sexual orientation and prevalence and recalled time point of changes in their sexual orientation.
Results
FtM were most frequently heterosexual, according to their gender identity, while MtF more often reported to be homosexual (72.5 vs 29% and 33.8 vs 7.5% respectively; $P<0.001$). MtF reported change in sexual orientation in total in 32.8% of cases in contrast to FtM with only 17.5% ($P=0.067$). 6 MtF (21.4%) reported change in sexual orientation without any sex reassignment surgery in their history in contrast to only 2 FtM (12.5%). Of those who had undergone sex reassignment surgery, most but not all subjects had experienced changes in their sexual orientation following the surgical procedure (13 MtF (73.3%), 5 FtM (71%)). While there was no significant difference seen in terms of time interval between date of introduction of cross-sex hormone treatment and changes in sexual orientation, MtF experienced changes in sexual orientation more quickly after sex reassignment surgery in contrast to FtM (13.8 vs 1.75 years $P<0.05$).
Conclusion
In contrast to earlier reports, we showed that changes in sexual orientation do not solely occur in the context of sex reassignment surgery. Furthermore, there are clear differences between MtF and FtM, who especially reported a significantly longer interval from sex reassignment surgery to the change in sexual orientation.

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P334

Somatostatin receptor sst2 transfer in somatolactotroph cell line. Constitutive activity and drug sensitivity

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Somatostatin is a ubiquitous neuropeptidic inhibitor of various cellular functions including endocrine and exocrine secretion. Moreover, this peptide may control cell proliferation in normal and tumoral tissues. Somatostatin biological effects are mediated by five subtype of G protein-coupled receptor, sst1 through sst5. The somatostatin analogs have high affinity for sst2 receptor and the success of *in vivo* peptide therapy is correlated with high levels of sst2 in the adenomas.
We previously demonstrated the re-sensitivity of human somatotroph adenomas to a somatostatin analog (octreotide) by sst2 expression after adenoviral transfer in adenomas cells *in vitro*. A ligand-independent activity of sst2 was also demonstrated in human adenomas in this study through an increase in the mortality and a decrease in secretion. Several GPCRs have been shown to exhibit varying degrees in ligand-independent activity including sst2 in a pancreatic adenocarcinoma models and in a corticotroph context.
To investigate more specifically the sst2 ligand-independant and ligand-dependant activity and their impact on cell physiology as proliferation and secretion in somatolactotroph context, an sst2 expressing cell line was generated after lentiviral transfer in the rat somatolactotroph cell line (GH4C1) and compared *in vivo* and *in vitro* to a eGFP-GH4C1 cell line. Sst2 mRNA level, sst2 immunostaining and somatostatin binding sites demonstrated a strong sst2 expression in this cell line. The level of somatostatin binding was closer to that found in human somatotroph adenomas.
A ligand independent impact of sst2 was observed on cAMP level, ERK activity, hormonal secretion and, to a lesser extent, on cell viability in early proliferation step, *in vitro* and *in vivo*.
Moreover, the octreotide sensitivity was consistently improved *in vitro* and *in vivo*, after sst2 transfer.
In conclusion, we have validated a somatotroph cell line, a useful tool to evaluate *in vitro* and *in vivo*, new somatostatin analogs.

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P335

The distinctive roles of thyroid hormone receptors in the development of zebrafish tissues

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Introduction
As in humans, thyroid hormone (TH) plays an essential role on zebrafish development by acting through the specific nuclear receptors (TRα or TRβ) and activating/suppressing the expression of several target genes. In zebrafish, TRα is preferentially expressed in the heart and CNS while the TRβ is mainly expressed in the retina and inner ear, but the specific role of these receptors in the different target tissues is presently unknown. In this study, we tested the role of TH and TRs during Zebrafish development and a particular attention has been devoted to the examination of the following target tissues brain, heart, sensory organs (retina and otoliths).
Methodology
Selvatic and transgenic zebrafish lines are injected with morpholinos, to knockdown the expression of the TRs and producing two different defective lines, MOs_TRα and MOs_TRβ. The morphological and functional anomalies of MOs_TRs were compared with MOs_ctrl at several time-points using different techniques: whole mount *in situ* hybridization, immunohistochemistry, histologic sections and confocal microscopy.
Results
Both defective morphant-fish lines display a series of growth alterations that depend on the type of defective-receptor and the preferential expression of the TR at tissue-level. i) Brain development: MOs_TRα show a severe cerebral hypoplasia and edema that impairs the proper CNS formation, while MOs_TRβ are quite comparable with controls. ii) Cardiac function: both MOs lines show structural and functional alterations compared to MOs_ctrl. MOs_TRα heart is heavily hypotrophic and bradycardic whilst MOs_TRβ are hypertrophic and tachycardic. iii) Sensory organs: only MOs_TRβ display severe abnormalities both in photoreceptors and otoliths (corresponding to the mammal innear ear) formation.
Discussion
Zebrafish is an excellent model to study the role of THs and TRs during embryonic development, representing an interesting and new *biotool* to test human TRα and TRβ mutations.

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P336

Effects of pharmacological and genetic manipulation of glucocorticoids during early development of the zebrafish embryo

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Background
The effects of glucocorticoids (GC) on the developing zebrafish embryo (Zfe) are poorly characterised. We have assessed the effects of pharmacological and genetic manipulation of cortisol and glucocorticoid receptors (GR) on global development and stress response during the first 120 hpf.
Methods
Cortisol production was modulated by inhibiting the enzyme 11b hydroxylase using morpholino gene knockdown (MO) or incubation in the drug metyrapone (Met) (10 μM). Downstream effects of GC were modulated using the GR agonist dexamethasone (Dex), GR antagonist RU486 or GR knockdown using targeted MO (GR-MO). The effects of these modulations on global development (head–trunk angle, growth-rate and swim bladder inflation), hatch-rate, whole embryo cortisol (WEC) levels, stress response (to stirring) and spontaneous swim behaviour were assessed.
Results
Global development was delayed by approximately 8 h following inhibition of GR mediated effects (Ru486 and GR-MO) but accelerated by Dex, this alteration was more apparent in the first 72 hpf with catch-up in growth by 96 hpf. Spontaneous hatching from the chorion was also delayed by inhibition of GR and accelerated by Dex. Inhibition of GR also resulted in altered swim behaviour.
Cortisol levels (WEC) increased following stress in control embryos at 72 hpf but not at earlier stages 36 hpf (6.7 ± 0.008 ng/embryo compared to 0.709 ± 0.16 ng/embryo respectively). This rise in WEC could be abolished with Met and MO treatment at 72 hpf but was unaffected at 36 hpf suggesting inactivity of this pathway at earlier developmental stages. Met and MO treated Zfe displayed abnormal post stress swim patterns, with reduced motility following stress compared to controls.
Conclusions
Stress response appears to be established in the Zfe by 72 hpf and can be modified by pharmacological and genetic manipulation of GC pathways without impairing global development significantly. These modifications result in altered hatching rates and swim behaviour.

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P337

A study on the relationship between energy reserves and energy expenditure during the time of male puberty

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Leptin, a key metabolic signal controlling both energy intake and energy reserves, informs the brain about energy stores of the body to initiate reproductive processes at puberty. Thyroid hormones are important determinant of overall energy expenditure, basal metabolic rate and thermogenesis. The present study examined the relationship between leptin and thyroxine (T_4) and triiodothyronine (T_3) in boys ($n=540$) between the age of 1 and 20 years. Blood samples were collected and plasma concentrations of leptin, T_4 and T_3 were measured using specific ELISA. Data were analyzed using Student's *t*-test, ANOVA and Pearson correlation *r*. Concentrations of leptin increased from 8th year, peaked at 10th year and gradually declined to lowest concentrations at 18th year. Concentrations of T_4 were higher at 1st year, increased to highest concentrations at 2nd, declined to lowest level at 4th and rose to peak at 5th year. T_4 concentrations declined from 9th year to lower levels between 11th and 13th year, rose to peak at 15th year and declined to lower levels between 16 and 20 years. T_3 concentrations exhibited a pleatu from 1 to 3rd year, declined to lowest levels at 4th year and gradually increased at 6th year to be maintained by 8th year. T_3 concentrations rose at 9th year, progressively increased to peak at 17 years, slightly declined at 18th years to be maintained by 20th year. Leptin and T_4 concentrations were positively correlated at infancy and prepuberty, negatively correlated at early puberty and positively correlated at mid and late puberty/adolescence. Leptin and T_3 concentrations were positively correlated at infancy, negatively correlated at prepuberty, positively correlated at early puberty and negatively correlated at mid and late puberty/adolescence. In conclusion, concentrations of leptin, an indicator of energy reserves, are negatively correlated with T_3, a marker of energy expenditure at mid and late puberty/adolescence.

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P338

TurboFlow-LC-MS/MS method for quantification of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione and testosterone in children

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Diagnosis and management of infants and children with sex steroid disorders requires fast and simultaneous assessment of several sex steroid metabolites in serum at low concentrations and on small sample volumes. Therefore, we developed a sensitive and selective TurboFlow-LC-MS/MS method for quantification of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione and testosterone in serum from pre-pubertal children.
Total run time was 10.75 min with the effluent being directed to the mass spectrometer for 2.6 min. Limits of quantification were determined as described by the International Conference on Harmonisation (ICH) with the following values: DHEA, 0.88 nM; DHEAS, 48 nM; 17α –hydroxyprogesterone, 0.19 nM; Δ4-androstenedione, 0.18 nM and testosterone, 0.10 nM. Intra-day relative S.D. ranged from 4.6 to 13.8% and inter-day relative S.D. ranged from 5.7 to 15.7%. Steroid concentrations in 186 serum samples from children (8.4–14.8 years old) were compared with results obtained by immunoassays for DHEAS, Δ4-androstenedione and testosterone. DHEAS and testosterone gave overall similar results with mean values 19 and 18% higher, respectively by LC-MS/MS, while levels of Δ4-androstenedione on average were found to be 83% higher when analysed by immunoassay. DHEAS was quantified in all samples with both methods, while Δ4-androstenedione and testosterone were quantified in 78 and 61% of the samples, respectively using immunoassay and in 98 and 94% of the samples, respectively using the LC-MS/MS method. Concentrations of the five steroids determined by LC-MS/MS were similar to previously published results. The presented method is suitable in a clinical setting for simultaneous quantification of five steroids important for management of children with disorders of sex development and steroid biosynthesis defects. Our study illustrates the importance of LC-MS/MS technology for quantification of – at least – Δ4-androstenedione and testosterone at low levels in children as an alternative to conventional immunoassays.

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P339

The prevalence, incidence and diagnostic delay in 46,XY females; a Danish national cohort study

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Introduction
Disorders of sex development where females have a 46,XY karyotype can be seen. Main reasons for this are the conditions of androgen insensitivity syndrome (AIS) and gonadal dysgenesis. The clinical phenotype of both conditions is variable and can present from an undervirilized or infertile male to an individual with ambiguous genitalia at birth, to a pure female phenotype with unambiguous genitalia, who first present in adolescence with primary amenorrhoea and/or delayed puberty. The aim of the study was to estimate prevalence, incidence and diagnostic delay in 46,XY females in an unselected population in a nationwide study.
Design
A retrospective cohort study.
Patients and methods
From the Danish Cytogenetic Registry data of all cases registered as females and diagnosed with 46,XY or a related male karyotype in Denmark during 1965–2010 were retrieved. Cases were divided into subgroups of females having a 46,XY karyotype, mosaicism (45,X/46,XY; 46,XX/46,XY) and 'other karyotypes'.

Information of the background population was retrieved from Statistics Denmark.
Results
Age at diagnosis was mainly distributed in two periods with 29% diagnosed within the first year of life and 38% diagnosed during adolescence (13–20 years). Median age at diagnosis was 11.6 years (range: 0–46 years) and age at diagnosis increased significantly during the study period ($P=0.005$). There was no difference in age at diagnosis comparing the subgroups (Kruskal–Wallis Rank Sum = 0.62). A prevalence of approximately six cases per 100 000 was observed during 1906–2010. During 1971–1990, the highest prevalence was observed with 13 cases per 100 000. From 1996 and forward the prevalence decreased.
Conclusions
Females with 46,XY and related male karyotypes are diagnosed with considerable diagnostic delay. Time trend in age at diagnosis shows increasing age at diagnosis during the study period. The prevalence of 46,XY female is higher than previously reported.

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P340

Effects of prenatal antiandrogen exposure on HSD3B expression in the fetal porcine gonads

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3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (HSD3B) is a key enzyme catalyzing an essential step in the formation of all classes of steroid hormones. Previously, we have reported the presence of androgen receptors in the fetal porcine gonads denoting the role of androgens during gonadal development. There is also growing evidence that steroid hormones modulate HSD3B expression. Thus, the aim of the present study was to determine the effect of androgen deficiency during late prenatal period on HSD3B expression in the fetal porcine gonads.
Pregnant sows were injected with anti-androgen flutamide (50 mg/kg bw, seven times, every day) starting at gestational day (GD) 83 or 101. The fetal gonads were obtained on GD90 or GD108. HSD3B immunolocalization was performed using rabbit polyclonal anti-mouse HSD3B antibody (provided by Prof. A H Payne from Stanford University). To assess HSD3B mRNA expression real-time PCR was carried out using the TaqMan Gene Expression Assay (Applied Biosystems).
In testes from control and flutamide-exposed fetuses, HSD3B was immunolocalized in Leydig cells. Following flutamide treatment, the number of HSD3B positive Leydig cells was higher on GD90 and lower on GD108 vs control. Moreover, flutamide administration resulted in increased HSD3B mRNA expression on GD90 and decreased HSD3B mRNA expression on GD108.
In ovaries from control and flutamide-exposed fetuses, HSD3B was immunolocalized in granulosa cells of forming follicles. Following flutamide administration, increased expression of HSD3B mRNA and protein were observed on GD90. However on GD108, flutamide treatment led to decreased HSD3B mRNA expression, while no changes in the intensity of immunostaining were observed.
In summary, diminished androgen action in porcine fetal gonads during late gestation induce changes in HSD3B expression, which may result in functional changes in Leydig or granulosa cells. However, it seems that androgens exert diverse biological effects depending on the gestational period.
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P341

Characteristics of the HIV positive transgender population of Catalonia

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Introduction
Previous studies indicate a high prevalence of HIV infection in the transgender population and associate it with sexual risk behavior, psychiatrical and socio-economic problems. Anyway, few data are available regarding other factors. We assessed the prevalence of HIV and other associated characteristics in a transgender population of Catalonia.
Methods
Cross-sectional study including 234 transsexuals that started follow-up in a reference center between 2006 and 2010. Socio-demographic, clinical, anthropometrical and analytical data were collected from the medical history at baseline and after 2 years of follow-up.
Results
The prevalence of HIV was 8.5%, higher in the male-to-female than in the female-to-male transsexuals (12.6 vs 2.2%). The HIV infection was more frequent in immigrants from South/Central America (20.8%) and other countries (14.3%) than in Spanish transsexuals (4.6%), $P<0.001$. There was no association with homosexuality, educational level and tobacco, alcohol or cannabis consumption. The prevalence of HIV was higher in transsexuals with a history of prostitution (26.5 vs 3.4%, $P<0.001$), cocaine consumption (20 vs 5.3%, $P=0.001$), heroin consumption (33.3 vs 7.5%, $P=0.009$), VHC infection (55.6 vs 7.3%, $P<0.001$), VHB infection (50 vs 6.4%, $P<0.001$), previous hormonal treatment (17.1 vs 1.6%, $P<0.001$) and sex reassignment surgery (17.5 vs 4.8%, $P=0.003$). The HIV+ transsexuals had higher levels of aspartate transaminase (32.1 vs 22.5 U/l, $P<0.001$), alanine transaminase (36.1 vs 23.05, $P=0.026$), gamma-glutamyl transpeptidase (131.6 vs 64.4, $P<0.001$), alkaline phosphatase (192.6 vs 97.09, $P<0.001$), higher triglycerides (102.4 vs 81.1, $P=0.019$) and lower HDL-cholesterol (39.5 vs 48.04, $P=0.004$). At 2 years of follow-up there were no differences in analytical values between HIV+ and HIV− transsexuals.
Conclusion
In this transgender community, the HIV infection was associated with the immigrant status, prostitution, cocaine and heroin consumption, VHC and VHB infection, previous hormonal treatment, sex reassignment surgery. Transsexuals VIH + had higher liver enzymes levels and worse lipid profile.

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P342

Optimising strategies for face classification in the detection of acromegaly

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Introduction
It has been shown that face classification software might help distinguishing between subjects with and without acromegaly on regular photographs. In this project, we investigated several aspects that will be necessary and helpful to bring this recognition method closer to clinical application.
Methods
Face classification was based on nodes placed on frontal and side photographs of individuals and analysis the underlying texture and geometric functions. In the first step, we analysed whether omission of nodes considered less relevant would change classification rates in the original database on 57 acromegalics and 60 controls. In a second step, we analysed how a completely new set of nodes (referring to the most common changes in morphological changes in face) will affect the classification rate.
In a third step, we analysed whether classification was improved in an external data set consisting of 82 acromegalics and 141 controls for both steps.
Results
Correct classification rates in the original database were 79% with all nodes 78% if irrelevant points were omitted and 80% using the new set of nodes.
Using the same approach, in the validation set, correct classification rates were 78% with all nodes (80 and 76% of acromegalics and controls, respectively) 86% (85 and 86% of acromegalics and controls, respectively) after omission of irrelevant nodes and 93% (92 and 93% of acromegalics).
Conclusions
Reduction of nodes associated with unwanted noise can improve correct classification rates in the detection of acromegaly by face classification software.

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P343

Evaluation of adults with phenyketonuria from pediatric to adult care

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Objective
Transition from pediatric to adult health care is a particularly vulnerable period

for patients with inborn metabolic diseases.
Our objective in this study was to evaluate the current transition of patients with phenylketonuria (PKU) in Malaga, Spain, by analysis of the metabolic control, medical care, patients satisfaction and phychosocial status.
Material and methods
We evaluate all the patients transferred to our adult unit between 2008 and 2012. Pediatric case notes and the present physician's case notes were analyzed retrospectively.
Results
Sixteen patients (8 F/M) patients were analysed. At the time of transition, 13/16 were in good metabolic control according to current treatment guidelines. 13/16 were on a low phenylalanine diet in combination with intake of a phenylalanine free amino acid mixture. 3/16 were taking BH 4 with normal intake of proteins. 6/16 had BMI higher than 25 kg/m^2 (1/16 with BMI$>$40). 1/16 had osteopenia and 2/16 had severe osteroporosis. 8/16 carried a secondary school certificate and 3/16 had achieved universitary studies. 3/16 with phychopatology (all of them with late diagnosis). 3/16 were married (two women had children).
Conclusions
The patients were quite satisfied with the transitional process. During transition medical care and metabolic control were stable. The individual optimization of the therapy, established during pediatric care provides the decisive ground work for disease control in adults. It's important to prepare, coordinate and evaluate transitional processes.

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P344

Resistant hypertension: about 30 cases

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Resistant hypertension (RH) is defined as blood pressure above a goal despite adherence to at least three optimally dosed antihypertensive medications of different classes, one of which is a diuretic.
Objectives
We investigated the prevalence of true resistant hypertension in our Department of Endocrinology, and we described the clinico-biological features of theses patients.
Methods
We performed a retrospective descriptive study. True resistant hypertension was diagnosed when white coat phenomenon, lack of compliance and secondary hypertension were excluded in patients with blood pressure $\geq$140/90 mmHg in two consecutive visits, despite to be using three blood pressure-lowering agents, including a diuretic.
Results
The sample included 30 patients (20 womens and 10 mens) among 563 patients followed in our department for hypertension.
The mean age of our patients was 54 years. Fifty percent were aged more than 65 years. Dyslipidemia was noted in 50% cases, and diabetes mellitus in 60% cases. Etiological investigation included an exploration of the aldo renin axis maded in 4, Hyperadrenocorticism in 9 cases, urinary metanephrines in 6 cases, thyroid tests in 17 cases, renal ultrasound in 9 cases, a Doppler ultrasound of the renal arteries in 8 cases. This assessment concluded that a primary aldosteronism was founded in 3 cases, an hyperthyroidism was in 1 case and hyperadrenocorticism in one case. Complications associated with this (RH) were hypertensive retinopathy in 8 cases (26.6%), nephropathy in 6 cases (20%) and left ventricular hypertrophy in 7 cases (23.3%). Diuretic therapy was prescribed in 100% of cases, angiotensin-converting-enzyme inhibitor in 66.6%, an angiotensin II receptor bloker in 26.6%, a calcium channel blocker in 76.6%, a B-blocking agents in 46.6%, an alpha blocker in 13.3%, centrally acting antihypertensive agents 30% and a spirinolactone in 3 cases. One patient underwent a right adrenalectomy with pathological diagnosis concluded an adrenocortical tumor.
Conclusion
Resistant hypertension is quite common. It is mainly due to the age. After confirmation of the diagnosis, it requires a thorough etiologic investigation and appropriate management.

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P345

High blood pressure in young adults: 84 cases

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Younger people with high blood pressure are 28% less likely to be diagnosed than older people. Arterial hypertension warrants further investigations to exclude secondary causes of hypertension in young people (aged $<$40 years).
Objectives
We conducted a retrospective descriptive study to investigate the characteristics hospitalized hypertensive young adults ($<$40 years) in our department.
Results
The sample included 84 patients: 25 mens (29.8%) and 59 womens (70.2%). A familial history of hypertension was noted in 67.8% cases. Among all patients, headaches (30%) were the most common presenting symptom, followed by palpitations in (24%) and flushing in (2%), but the diagnosis of hypertension was fortuitos in (46%). The complications of the hypertension included hypertensive nephropathy in (23%), retinopathy in (10%), transient ischemic stroke in (7.1%) and left ventricular hypertrophy in (13%). The investigations concluded to an essential hypertension in the majority of cases (79.8%). Secondary hypertension included obstructive sleep apnea syndrome (7.1%), a primary aldosteronism (3.6%), Cushing's syndrome (2.3%), a phéochromocytoma (1.19%), renal artery stenosis (2.3%), Basedow's desease (1.19%), corticosurrénaloma (1.19%), macronodular adrenocortical hyperplasia (1.19%).
Conclusion
Hypertension in young adults is increasing in frequency. While classic teaching dictates that secondary causes are more common in young adults, rates of essential hypertension are progressively rising. This can be explained by the high incidence of metabolic syndrome in young adults.

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Diabetes

P346

Elevation of circulating free fatty acids abolishes down-regulation of skeletal muscle adiponectin receptor 1 (AdipoR1) expression caused by insulin infusion

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Introduction
Adiponectin is an adipocytokine with insulin-sensitizing and anti-inflammatory properties. In skeletal muscle, it increases fatty acid oxidation and insulin-stimulated glucose uptake through binding with adiponectin receptor 1 (AdipoR1). The aim of our study was to assess the effect of insulin and Intralipid/heparin infusions on muscle AdipoR1 expression in humans.
Methods
Twenty healthy male subjects (age 25.2$\pm$3.2 years; BMI, 26.5$\pm$4.6 kg/m^2) with normal glucose tolerance were studied. Six-hours euglycemic hyperinsulinemic clamp was performed two times: with and without concurrent Intralipid/heparin infusion. Indirect calorimetry was performed at baseline and every 2 h of the clamp. Biopsy of vastus lateralis muscle was performed before and after each clamp. Muscle AdipoR1 and insulin receptor substrate 1 (IRS1) mRNA expression was analyzed with Real Time PCR.
Results
Intralipid/heparin infusion resulted in a decrease in insulin sensitivity by $\sim$40% ($P<0.0001$). Serum adiponectin concentration decreased similarly during both clamp (~-25%, both $P<0.001$). Muscle AdipoR1 was positively related to serum adiponectin ($r=0.46$, $P=0.046$), insulin sensitivity ($r=0.50$, $P=0.023$), muscle IRS1 ($r=0.55$, $P=0.014$), but negatively to respiratory quotient in insulin-stimulated conditions ($r=-0.45$, $P=0.046$). Insulin infusion decreased muscle AdipoR1 expression by $\sim$30% ($P=0.006$). This effect was almost completely abolished by concurrent Intralipid/heparin infusion (-7%, $P=0.44$;

the difference between two experiments, $P=0.027$).
Conclusions
Our data indicate that elevation of circulating free fatty acids abolishes down-regulation of muscle AdipoR1 expression caused by insulin, independently of the changes in serum adiponectin. This may be a potential compensatory mechanism for free fatty acid-induced insulin resistance.

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P347

Short stature, low-set ears, short 5.finger, undescended testis, bradycardia

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A 22-year-old man presented to the Endocrinology unit for short stature. His height is 1.53 cm; weight is 38.5 kg. Physical examination revealed short stature, lean body mass, low-set ears and a short fifth finger on the right. His bone age were consistent with 19-year. He had a history of undescended testis operation at 5-year old and history of strabismus surgery at 14-year old. He had a delta f 508 heterozygote mutation with normal sweat test. His biochemical analysis except total and direct bilirubin, thyroid hormone levels were within normal limit. Thrombocytopenia was occurred in the analysis of complete blood count. Platelet count was 113 000/ml. Basal cortisol level was obtained as 455 nmol/l. Serum FSH and LH levels were found to be increased. The FSH level was 38.4 mIU/ml (1.5–12.4) and the LH level was 15.37 mIU/ml (1.7–8.6). The serum level of somatomedin-C level was within normal limit for age. A sperm analyses revealed azoosspermia with 3 cc amount of semen, 3–4 leukocyte and 8.2 pH. He had normal thyroid and whole abdomen ultrasonographic findings. His scrotal imaging was as follows; right testis was not visible due to operation, left testis parenchyma was heterogeneous and size was $32\times14\times34$ mm with no mass imaging. The serum level of total testosterone was obtained as 5.34 ng/ml (2.49–8.36) within normal limit. His ECG findings revealed a sinus bradycardia and incomplete right bundle branch block. Floppy mitral valve within 67% ejection fraction was observed in echocardiographic evaluation. Osteoporosis was obtained in bone mineral density measured by dual-energy X-ray absorptiometry. Injectable testosterone esters were given. After 1 month of testosterone therapy, the FSH level was decreased to 30.47 mIU/ml and the LH level was decreased to 10.23 mIU/ml and the testosterone level was 5.65.

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P348

Long-acting insulin analogs exposure and cancer specific mortality in patients with diabetes mellitus

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Aim
To test the hypothesis that exposure to insulin glargine might be associated with increased risk of cancer mortality compared with human basal insulin preparations.
Materials and methods
All consecutive diabetes patients aged over 40 years, residing in a major urban area were screened at their first diabetes outpatient visit between 01/01/2001 and 12/31/2008 ($n=79\,869$). Exclusion criteria were insulin treatment at screening ($n=14\,752$), no insulin treatment until 12/31/2008 ($n=55\,795$), <6 months of glucose-lowering treatment alone before insulin initiation ($n=1154$), insulin prescription before glargine became available (04/17/2003, $n=1761$), age <40 or ≥80 years at first insulin prescription ($n=406$), <6 months of insulin treatment following insulin initiation. A total 4990 subjects were followed-up for death based on death certificate, until 12/31/2011, using data from National Institute of Statistics. Baseline was defined at 6 months after insulin initiation. Antidiabetic prescriptions were available. Adjusted time-dependent competing risk regression analysis, with daily updates of treatment modalities was performed. Simultaneously use of cumulative exposure and ever exposed term of the available treatment options, a 'fixed' cohort, cumulative exposure limited to that attained one year prior to death (minimizing the reverse causation), and a propensity score analysis completed the evaluation.
Results
Mean baseline age was 62 ± 9 years, and follow-up 4.7 ± 1.9 years (23 179 person-years). Glargine cumulative dose exposure significantly lowered cancer mortality risk, subhazard ratio (SHR) 0.94 (95% CI 0.89–0.99, $P=0.033$). Cumulative exposure limited to that attained 1 year prior to death showed a glargine cumulative exposure time SHR 0.94 (95% CI 0.89–0.99, $P=0.018$) and cumulative dose SHR 0.92 (95% CI 0.86–0.98, $P=0.014$). Glargine use was associated with cumulative exposure time and cumulative dose that were significant predictors for lower pancreatic and breast cancer mortality, but with no impact on lung, colorectal, female genital, liver, and urinary tract cancer deaths.
Conclusions
The cumulative dose exposure to insulin glargine was associated with a lower risk of cancer mortality in general, and of breast and pancreatic cancer in particular. No glargine associated 'harm' was found even after additional 'fixed' cohort or propensity score analyses were performed.

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P349

Expression of adiponectin gene and adiponectin receptors in placental and adipose tissue in women with gestational diabetes mellitus

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Introduction
Adiponectin shows its beneficial insulin-sensitizing properties, however, women with gestational diabetes mellitus (GDM) present hypoadiponectinemia.
Aim of the study
To assess the expression of adiponectin gene as well as its AdipoR1 and AdipoR2 receptors in placenta and adipose tissue and to determine potential correlations with chosen metabolic parameters (clinical and biochemical).
Patients and methods
Thirty-six pregnant women, who for obstetric reasons underwent cesarean sections, were divided into two groups. The study group included 20 women diagnosed with GDM by routine prenatal tests, whereas the control group comprised 16 women with normal oral glucose tolerance test (OGTT). Intraperitoneal and subcutaneous adipose tissue samples were obtained intraoperatively from all women, whereas the placental samples were obtained straight after delivery. Adiponectin, AdipoR1 and AdipoR2 gene expressions were assessed using the r-tPCR method.
Results
Serum glucose and insulin concentrations, HOMA-IR and HOMA-B levels were significantly higher in the GDM group, whereas the QUICKI values were respectively lower. Adiponectin concentrations were significantly lower in the study group than in the control one 4.9 ng/ml (4.4–5.8) vs 5.9 ng/ml (5.1–8.0 ng/ml). The expression of AdipoR1 receptor was significantly higher in the study group, whereas there was no significant difference between the expression of adiponectin and AdipoR2 receptors between the groups.
Conclusion
Impaired glucose tolerance in women with GDM modifies the expression of AdipoR1 in placenta and adipose tissue, but it does not affect the level of expression of adiponectin mRNA and AdipoR2.

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P350

Effects of Vaspin on expressions of NF-κB and its target genes in endothelial EA.hy926 cells

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Introduction
The visceral adipose tissue-derived serine protease inhibitor (vaspin), a novel adipocytokine, has been widely reported to be associated with obesity and insulin resistance. Obesity induced chronic inflammatory process plays a pivotal role in insulin resistance and is characterized by the activation of NF-κB pathway and its target genes. The present study aims to investigate the effects of vaspin on NF-κB pathway and its target genes in basal and TNF-α stimulated endothelial EA.hy926 cells to further elucidate the role of vaspin in the development of obestiy and insulin resistance, which may provide diagnostic and therapeutic strategies in the treatment of obesity and insulin resistance correlated disorders, such as type-2 diabetes and metabolic syndrome.
Materials and methods
In this study, we constructed a NF-κB luciferase reporter system and stably transfected endothelial EA.hy926 cells with reporter plasmid pNF-κB-luc. Following transfection, basal and TNF-α stimulated EA.hy926 cells were treated with various concentrations of vaspin (0–3200 ng/ml). Luciferase activity assay was used to determine the transcription activities of NF-κB. Expressions of target genes-ICAM-1, VCAM-1 and MCP-1 were measured by real-time fluorescence quantitative PCR and western-blot in mRNA and protein levels.
Results
Results showed that vaspin significantly activated the expression of NF-κB in transfected EA.hy926 cells in a dose-dependent manner ($P<0.05$). Levels of ICAM-1, VCAM-1 and MCP-1 were significantly increased by the treatment of EA.hy926 cells with vaspin (>200 ng/ml) or TNF-α (10 ng/ml), which can both be obviously reversed by the use of NF-κB inhibitor Bay 11–7082 (10 μM) ($P<0.05$). However, vaspin inhibited the TNF-α induced activation of NF-κB in a dose- and time- dependent manner ($P<0.05$).
Conclusions
Vaspin activated the expression of NF-κB and its target genes but inhibited TNF-α induced activation of NF-κB, indicating a dual role of vaspin in NF-κB pathway in human vascular endothelial cells and a potential mechanism in the regulation of inflammation in insulin-resistance.

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P351

Skeletal muscle and adipose tissue glycoprotein 130 expression is associated with insulin resistance in humans

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Introduction
Glycoprotein 130 (gp130) cytokines, like interleukin 6 (IL6), act through plasma membrane receptors consisting of two glycoproteins: a cytokine binding subunit (like IL6R) and gp130, responsible for signal transduction. IL6 may regulate insulin sensitivity in a dual manner, as both insulin-sensitizing and -desensitizing actions have been reported. Other gp130 cytokines may have a beneficial metabolic effects. The aim of the present study was to assess skeletal muscle and adipose tissue IL6R and gp130 expression in relation to insulin sensitivity and obesity in male subjects.
Methods
We examined 86 young (age 23.22±2.41 years), apparently healthy male subjects with normal glucose tolerance, 45 lean (BMI below 25 kg/m^2) and 41 with overweight or obesity (BMI between 25 and 40 kg/m^2). Euglycemic hyperinsulinemic clamp, indirect calorimetry and biopsies of vastus lateralis muscle and subcutaneous adipose tissue were performed. Tissue mRNA expression of IL6R, gp130, suppressor of cytokine signaling 3 (SOCS3), AMP-activated protein kinase (AMPK), and nuclear factor κ B (NFκB) was analyzed with real-time PCR.
Results
Obese subjects had higher adipose tissue gp130 expression ($p=0.008$). No difference in tissue IL6R was found. Both muscle and adipose tissue gp130 expression was inversely related to insulin sensitivity ($r=-0.32$, $P=0.004$ and $r=-0.28$, $P=0.035$, respectively). Only adipose tissue gp130 was related to its serum soluble form ($r=0.34$, $P=0.01$). Muscle gp130 was related to lipid oxidation during the clamp ($r=0.24$, $P=0.035$) and muscle AMPK ($r=0.48$, $P=0.042$). Adipose tissue gp130 was associated with adipose tissue SOCS3 ($r=0.60$, $P=0.007$) and NFκB expression ($r=0.49$, $P=0.035$).
Conclusions
Our data show that both muscle and adipose tissue gp130 expression is inversely related to insulin action. This association may be linked to lipid oxidation in muscle and inflammation in adipose tissue.

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P352

Increased alanine aminotransferase levels and associated characteristics among newly diagnosed type 2 diabetes patients: results from the DD2 study

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Objectives
There is limited knowledge of high-sensitivity C-reactive protein (CRP) levels among newly diagnosed T2D patients in the population-based setting, and of factors associated with elevated CRP.
Methods
Measurements of CRP were performed in the first 1036 T2D patients in the nationwide DD2 project, enrolling new T2D patients from general practitioners and hospital specialist outpatient clinics. We examined the number of T2D patients within CRP tertiles and clinically relevant cutoff points of CRP (<1.0, 1.0–2.99, 3.0–5.99, or ≥6.0 mg/l), and demographic, clinical, and lifestyle characteristics associated with elevated CRP.
Results
Median CRP was 2.1 mg/l (inter-tertile range, 1.3–3.6 mg/l). 20.6% of patients had a CRP level of 3.0–5.99 mg/l and 19.6% had a CRP of ≥6 mg/l. As compared to the 361 people with CRP values in the lowest tertile (≤1.3 mg/l), those with CRP values in the highest tertile (>3.6 mg/l) were younger (median age 58 vs 62 years, $P<0.0001$) and more likely to be female (52.7 vs 39.3%, prevalence ratio 1.34 (95% CI: 1.18–1.50)). Patients in the highest CPR tertile had substantially higher median values of BMI (34 vs 28.7 kg/m^2, $P<0.001$) and waist circumference (113 vs 100 cm, $P<0.001$), and were less likely to do regular sports activities (30.8 vs 46.3%, prevalence ratio 0.67 (95% CI 0.47–0.86). They also had a higher median HbA1C (7.4 vs 6.6%, $P<0.001$), higher fasting blood glucose (7.26 vs 6.82 mmol/l, $P<0.0001$), higher C-peptide (784 vs 545, $P<0.0001$), and were more likely to be on insulin treatment (7.7 vs 5.0%, prevalence ratio 1.54 (95% CI: 0.96–2.13). Blood pressure, lipids, and current smoking were similar between groups. Patients with high CRP had higher Charlson comorbidity index scores (score ≥1 in 35.2 vs 26.1%) and more previous cardiovascular disease (20.4 vs 16.9%) and chronic pulmonary disease (12.4 vs 6.6%). Nonetheless, fewer in the elevated CRP group were on statin therapy (58.9 vs 69.3%).
Conclusions
Among newly diagnosed T2D patients in Denmark, 19.6% had CRP values of more than 6 mg/l. Patients in the highest CRP tertile were more likely to be female, obese, physically inactive, and comorbid, with worse blood glucose control compared to those with low CRP.

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P353

Autoantibodies to the insulin- and IGF1-receptor in human sera

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Introduction
Autoantibodies (aAB) are characteristic of autoimmune diseases, but may also be found in apparently healthy individuals and precede pathological symptoms. We have recently reported on autoantibodies against the IGF1-receptor (IGF1R-aAB) in control subjects and patients with Graves' disease (1). The isolated aAB were able to immunoprecipitate the recombinant autoantigen and antagonized IGF1 signaling *in vitro*. Given the structural similarities between IGF1R and insulin

receptor (IR), we decided to establish a diagnostic test for IR-aAB similar to our IGF1R-aAB assay. IR-aAB are of endocrine importance as they are capable of causing insulin resistance type B.
Objective
To compare the prevalence and potential cross-reactivity of IGF1R-aAB and IR-aAB.
Methods
Recombinant variants of both receptors were stably expressed in HEK293 cells and used to establish diagnostic autoantibody assays as described (1). A total of 400 samples were obtained from a commercial supplier and analyzed for IGF1R-aAB and IR-aAB presence. Cross-reactivity of positive sera was tested with recombinant IGF1R and IR.
Results
A comparable number of sera turned out to be positive for IGF1R-aAB and IR-aAB, respectively, with a prevalence of ~5% each. A fraction of positive samples turned out to react with both the IGF1R and the IR indicating either the presence of multiple specific-aAB isoforms or one type of cross-reacting aAB in a given serum. Clonality and cross-reactivity of the aAB identified are currently under investigation.
Conclusion
Our studies demonstrate an unexpected high prevalence of aAB against IGF1R and IR in human sera. In light of case reports on insulin resistance type B, these findings may be of clinical relevance for the present diabetes epidemy, but further studies are needed to test this hypothesis.
1. Minich WB, *et al. Journal of Clinical Endocrinology and Metabolism*, 2013 (in press, PMID: 23264397).
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Physiological role for Angptl4/fiaf in exercise-induced muscle AMPK activation

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Introduction
Angiopoietin-like protein 4 (Angptl4), also known as fast-induced adipose factor (Fiaf), is a glycoprotein which is known as a potent inhibitor of lipoprotein lipase. We have reported that hypothalamic Angptl4 is a novel anorexigenic molecule by inhibiting hypothalamic AMPK activity. In the present study, we investigated a potential role of Angptl4 in exercise-related physiology.
Methods
We determined changes in Angptl4 mRNA/protein levels along with phospho-total AMPK levels in leg skeletal muscle of mice following moderate strength prolonged running exercise (18 m/min for 50 min). We treated Angptl4 peptide in C2C12 skeletal muscle cells to test if Angptl4 can activate AMPK activity in skeletal muscle. Finally, we compared the effects of chronic running exercise (5–18 m/min for 50 min, five times/week for 2 weeks) on muscle AMPK activity and food intake of Angptl4 null mice and their wild littermates.
Results
Running exercise significantly increased Angptl4 mRNA and protein levels and AMPK phosphorylation in skeletal muscle. Treatment of Angptl4 in C2C12 cells significantly increased AMPK and ACC phosphorylation in a dose- and time-dependent manner. Exercise-induced AMPK activation in skeletal muscle was diminished in Angptl4 null mice. Chronic running exercise significantly reduced food intake in wild mice, but exercise-induced anorexigenic effect were blunted in Angptl4 null mice.
Conclusion
Our data have demonstrated a potential role of skeletal muscle Angptl4 in exercise-induced AMPK activation and exercise induced anorexia. Therefore, Angptl4 may mediate metabolic changes during exercise.
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P355

Predictive value of HbA1c and glucose fluctuations in hypoglycemia risk in patients with type 1 diabetes

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Introduction
Many patients with diabetes experience high variability in glucose concentrations which is associated with a risk of hypoglycemia. The aim of this was to compare the predictive value of HbA1C, mean interstitial glucose (MIG), and glucose variability (GV) in the risk of hypoglycemia in patients with type 1 diabetes (T1D).
Methods
Continuous glucose monitoring, using CGMS system, was performed in 130 T1D patients with diabetes duration of 17.1±8.6 years, in intensive insulin therapy (49.8±17.9 IU). MIG (in mg/dl), GV measured by standard deviation of MIG (mg/dl), time per day spent in hypoglycemia (HT), interstitial glucose ≤70 mg/dl (h), and episodes of nocturnal hypoglycemia (NH), hypoglycemia between midnight and 0800 h, in %, were assessed. Patients were divided in group I ($n=84$) with HbA1c ≤7.5%, and group II ($n=46$) with HbA1c >7.5%. Statistical analysis was performed using SPSS, version 21.0.
Results
Group I presented a significantly lower MIG (139.2±25.9 vs173.1±33.2 mg/dl, $P<0,05$) and GV (58.4±18.8 vs 70.3±18.6 mg/dl, $P<0.05$) and more HT (1.85±1.68 vs 1*P*30±1.04 h $P<0,05$). NH episodes weren't significantly different between groups (8.7 vs 4.8%). HT was positively correlated with GV ($r=0.23$, $P=0.01$) and negatively with HbA1C and MIG ($r=-0.23$ and $r=-0.36$; $P=0.01$). NH was correlated with MG ($r=-0.24$, $P<0.05$). In multivariate analyses, GV and MIG were associated with HT ($\beta=0.22$ and $\beta=-0.15$, $P<0.01$ respectively), independently of HbA1C, diabetes duration and insulin dose; NH was only associated with MIG (OR=0.9, $P<0.05$). NH was associated with 16-fold increased risk of asymptomatic hypoglycemia (OR: 16.9; $P<0.01$).
Conclusions
Group I patients presented lower MIG and GV, and more HT than group II. NH episodes weren't significantly different between groups. An elevated HbA1c wasn't a reliable indicator of lower risk of hypoglycemia. GV independently predicts daily time duration spent in hypoglycemia.
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Risk factors for impaired glucose tolerance and diabetes mellitus after liver transplantation

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Risk factors involved in new-onset diabetes mellitus after transplantation (NODAT) remain unclear. Clinical studies have demonstrated the association between low vitamin D levels with insulin resistance and diabetes. In animal models, osteocalcin deficiency leads to high glucose levels and insulin resistance. The aim of this study is to evaluate the prevalence of NODAT and other alterations of glucose metabolism after liver transplantation (LT) at our institution. Also, we assess the relationship between serum osteocalcin, 25-OH vitamin D levels and NODAT. Influence of hepatitis C virus (HCV) infection was also studied.
Methods
In the period 2011–2012, 133 LT patients (90 men and 43 women) with a mean age of 58.8 years were studied. None of them had been diagnosed of diabetes prior to transplant. Minimum period posttransplantation was 6 months and mean follow-up was 9.2 years. 75 g oral glucose tolerance test (OGTT) was performed to assess glucose, insulin and C-peptide levels at baseline, and 60 and 120 min. Data on medical history, anthropometric measurements and lab test (including HbA1c, total osteocalcin, and 25-OH vitamin D levels) were collected.
Insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI) indexes were calculated. 2011 ADA diagnostic criteria were used.
Results
Thirty four patients developed diabetes or were treated with insulin or oral antidiabetic medication. OGTT was performed in 99 patients: 12.1% had NODAT criteria, and 32.3% showed impaired glucose tolerance (IGT). 30.3% showed vitamin D deficiency (<20 ng/ml). No significant differences were observed in osteocalcin and vitamin D levels between NODAT, IGT, and normal. No correlation was found between osteocalcin and glucose, insulin, C-peptide, or HbA1c. HOMA-IR was significantly higher in diabetic ($P<0.001$) than in normals. QUICKI was significantly lower in prediabetic ($P<0.05$) and diabetic ($P<0.001$) compared with normal. HCV positive status (before and after transplantation) was associated with diabetes onset ($P=0.05$ and 0.001 respectively.
Conclusion
Prevalence of NODAT and IGT is high in LT recipients. Our study didn't find

osteocalcin and vitamin D levels as predisposing factors for the onset of both conditions. Hepatitis C virus infection increased the risk of NODAT.

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Acute exercise leads to increased HbA1c and fructosamine levels in athletes with type 1 diabetes

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Introduction
In patients with type 1 diabetes, exercise has not consistently been shown to improve glycemic control, as measured using HbA1c levels. Participation in competitive sports may even worsen glycaemic control. Free radicals and oxidative stress markers are known to increase during acute exercise in parallel to decreased circulating antioxidant concentrations. We aimed to analyze the effects of chronic and acute exercise on circulating HbA1c and fructosamine levels.
Design
We evaluated six patients (five men and one woman). Their mean age was 41.8 (range: 22–49). Their mean diabetes duration was 11.3 years (range: 5–20). All of them were under intensive treatment before the study (four with MID and two with ISCI). Prior to the training period, they usually ran 35 km 3 days every week, which increased to 70–80 km 5 days/week during the training period. We analyzed their HbA1c and fructosamine levels before and after the training period and also before and after running a half-marathon (20 km).
Results
After eight months training, mean HbA1c decreased from 7.80% (95% CI 6.80–8.79) to 7.55% (95% CI: 6.52–8.57) (P 0.13) and mean fructosamine levels decreased from 370.5 µmol/l (95% CI 311.87–429.12) to 350 µmol/l (95% CI 292.97–407.02) (P 0.075). Paradoxically, after acute exercise, mean HbA1c increased from 7.53% (95% CI 6.54–8.52) to 7.61% (95% CI 6.61–8.51) (P 0.025) in parallel to raised fructosamine levels from 348.3 µmol/l (95% CI 298.1–398.5) to 363.8 µmol/l (95% CI 318.3–409.2) (P 0.046). HbA1c was determined using HPLC (reference range: 4.1–6.2%, Intra and interassay coefficients of variation (CV) were 0.7 and 0.74% respectively). Fructosamine was determined by spectrophotometer (normal values $\leq$285 µmol/l. Intra and interassay CV were 1.7 and 3.9% respectively.
Conclusion
Acute exercise should be taken into account as one of the factors influencing HbA1c variability in a short time.

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Increased alanine aminotransferase levels and associated characteristics among newly diagnosed type 2 diabetes patients: results from the DD2 study

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Objectives
Elevated levels of serum alanine aminotransferase (ALAT) have been linked with non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), insulin resistance and the metabolic syndrome in type 2 diabetes (T2D) patients. We examined ALAT levels in newly diagnosed T2D patients, and the factors associated with such elevation.
Methods
Measurements of ALAT were performed in 1025 (43% women and 57% men) new T2D patients enrolled from general practitioners and hospital specialist outpatient clinics in the nationwide DD2 project. We calculated the median value of ALAT, and examined the number of T2D patients within gender specific quartiles of ALAT values. We also examined demographic, clinical, and lifestyle characteristics associated with increased ALAT levels. Data on BMI, lipid profile and blood pressure could be ascertained from the Danish Diabetes Database for Adults for a subgroup (n=525) of the study population.
Results
The median value of ALAT was 24 IU/l (inter-quartile range 18–32 IU/l) in women and 30 IU/l (inter-quartile range 22–41 IU/l) in men. 26% were in the lowest ALAT quartile ($<$18 IU/l for women and $<$22 IU/l for men, respectively) and 24% were in the highest ALAT quartile ($>$32 IU/l/$>$41 IU/l for women/men). As compared to people with ALAT values in the lowest quartile, those with high ALAT were younger (median age 57 vs 64 years, $P<0.0001$), more obese (median BMI 31.2 vs 29.1 kg/m^2, $P=0.004$), and had a larger waist circumference (111 vs 101 cm, $P<0.0001$) and higher median CRP levels (2.8 vs 1.8 mg/l, $P=0.0147$). They also had substantially poorer glucose control (HbA1c 7.40 vs 6.90%, $P=0.084$; fasting blood glucose 7.56 vs 6.86 mmol/l, $P<0.0001$), and a worse lipid profile (total-cholesterol 4.70 vs 4.15 mmol/l, $P=0.006$), whereas blood pressure was similar between groups. Patients in the highest ALAT quartile had more alcohol overuse (10.4 vs 2.2% with $>$14/21 weekly drinks in women/men, $P<0.0001$) as compared to subjects in the lowest quartile.
Conclusions
Among newly diagnosed T2D patients, those with a high ALAT level were younger, had more abdominal obesity, dyslipidemia, poorer glucose control, more alcohol overuse, and higher CRP levels as compared with those in the lowest ALAT quartile.

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Reconsidering guidance for postnatal glucose screening in gestational diabetes

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Background
National Institute for Health and Clinical Excellence (NICE) guidelines advise that GDM be diagnosed using a 2-hr 75 g oral glucose tolerance test (OGTT). However there is lack of consensus on the optimal *postpartum* surveillance. Current postpartum screening guidance suggests a single fasting plasma glucose (FPG) at 6-weeks with annual testing thereafter. As women with GDM are often from mixed ethnic groups and frequently have postprandial hyperglycaemia, a 2-h OGTT result may be more relevant.
Objectives
Our study assessed if postpartum screening using FPG alone missed significant numbers of patients with pre-diabetes or impaired glucose tolerance (IGT).
Methods
Retrospective analysis of 211 women diagnosed with GDM over a 4-year period was undertaken. All pregnant women had a plasma glucose screen at booking and 28 weeks. OGTT was undertaken if FPG was $>$6.0 mmol/l or random plasma glucose was $\geq$7 mmol/l, and also in the presence of glycosuria or major clinical risk factors. All patients were invited for a postnatal OGTT at 6-weeks *postpartum*.
Results
12 788 mothers were subject to plasma glucose screening in pregnancy. GDM was diagnosed on OGTT if FPG was $>$6.0 mmol/l or 2-h value was $\geq$7.8 mmol/l and the incidence was 1.65%. In 33 women a FPG $>$6.0 mmol/l was observed (7.62$\pm$0.76 mmol/l, mean$\pm$S.E.M.) and all patients had elevated 2-h values $\geq$7.8 mmol/l (9.79$\pm$0.01). 79% of women attended for postnatal OGTT. 6 women had frank diabetes and 12% had impaired glucose tolerance (2-h value 9.30$\pm$0.06 mmol/l) which would have been missed on FPG alone.

FPG (mmol/l)	2 hour plasma glucose (mmol/L)		
	< 7.8	7.8–11.1	≥ 11.1
≤ 16	141	16	–
6.1–6.9	–	4	1
≥ 7	2**	–	3*

*1 patient, FPG unknown, 2 hr 11.7mmol
**1 patient FPG 10.4, 2 hr unknown

Conclusion
GDM was diagnosed most commonly on elevated 2-h glucose rather than FPG. Using FPG alone for postnatal screening misses a substantial number of cases of

women with persisting IGT. As post-prandial hyperglycaemia is associated with increased cardiovascular risk, lifestyle advice and closer surveillance is required.

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TCF7L2 gene polymorphisms may influence insulin sensitivity and leptin levels independently from BMI and body fat content

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Introduction
The genome-wide association studies have recently expanded the number of genetic susceptibility loci for type 2 diabetes and obesity. Transcription factor 7-like 2 (*TCF7L2*) gene seems to be one of the most predictive identifiable factors promoting T2DM development. It has been suggested that TCF7L2 influences pancreatic β-cell function butthe effect of genetic variants of TCF7L2 on metabolic syndrome development is not well characterized among subjects with obesity. The aim of our study was to analyze whether genetic variants of the *TCF7L2* gene influence fasting leptin levels and insulin sensitivity in nondiabetic obese/overweight subjects.
Methods
We genotyped previously identified TCF7L2 SNPs: rs7901695 and rs7903146 in 944 subjects (463 women and 481 men), who underwent anthropometry (BMI) and body composition analysis: percent of body fat, visceral, and subcutaneous abdominal adipose tissue by multi-frequency bio-impedance method.
Results
In the present study we found that subjects with TT rs7903146 TCF7L2 homozygotes presented significantly higher fasting levels of leptin (29.7 vs 19.7 ng/ml, $P=0.035$) and higher HOMA-IR (3.9 vs 2.6, $P=0.009$), despite the lack of differences in BMI, body fat content, and body fat distribution. Moreover in the logistic regression analysis presence of CC genotype of rs7901695 TCF7L2 predicted higher IR independently from BMI, gender and caloric intake ($P<0.01$).
Conclusions
We believe that our study may help to understand the pathways that *TCF7L2* gene influence the risk of T2DM and provide personalized treatments and prevention strategies to fight against type 2 diabetes.

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P361

Type 2 diabetes and prostate cancer risk in Korean men with low PSA

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Introduction
Diabetes as a risk factor of cancer should be explored further. The value of PSA is limited because we cannot exclude prostate cancer in lower PSA levels. We analyzed diabetes and the risk of prostate cancer in lower PSA in a large cohort study.
Method
A total of 179 456 health examinees (men, aged 30–89 years) from 1994 to 2010 were participated. Participants with any cancer or potential prostate diseases were excluded from analysis. Prostate cancer incidence/death outcome was collected from hospital admission records due to prostate cancer from 2002 to 2010.
Result
Serum PSA measurements were divided into seven levels: Q1, 0–0.56, Q2, 0.57–0.79, Q3, 0.80–1.19, Q4, 1.20–2.50, Q5, 2.51–4.00, Q6, 4.01–10.00, and Q7, 10.01 ng/ml and above. The number of prostate cancer cases was 422 within Q1–Q4 and 594 in participants with PSA higher than 2.5 ng/ml. In Q5–7, the prevalence of diabetes was higher than Q1–4 (P for trend $<.0001$). In Cox proportional hazard models, participants in Q4 showed higher hazard ratios with prostate cancer incidence after adjustment for multiple confounding factors compared to the Q1 group, though they were within the usual normal range. Participants in Q3 and Q4 with PSA lower than 2.50 ng/ml showed significantly higher HRs (Hazard ratio (HR) 6.34, 95% CI 4.40–9.15 in Q4; HR 1.61, 95% CI 1.01–2.45 in Q3).
Conclusion
These findings suggest that examinees with lower PSA than 2.5 might need to be followed up, particularly in diabetes (This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, 14 245 and by the Korea government (MEST) (2011-0029348).

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P362

The association between severity of impaired glucose tolerance in gestational diabetes with age, BMI, and ethnicity in the UK

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Introduction
Gestational diabetes (GDM) has been shown to cause adverse fetal outcomes including macrosomia, shoulder dystocia and stillbirth. The multinational Hyperglycaemia and Pregnancy Outcome (HAPO) Study has shown there is a linear relationship between OGTT result and fetal growth. Investigating epidemiological risk factors is essential to ensure appropriate high-risk groups are being screened.
Methods and materials
A retrospective case note review was undertaken of all patients with newly diagnosed GDM ($n=321$) over a 2-year period at the West Middlesex University Hospital. The 75 g OGTT results were recorded with relevant demographic data including parity, age, BMI, and ethnicity.
Results
Asian ethnicity had the highest fasting plasma glucose ($\mu=5.49$; $n=208$), and 2-h plasma glucose ($\mu=9.32$; $n=208$). One-way ANOVA revealed a statistically significant difference between Caucasian, Black, Asian, and Oriental ethnicities with fasting glucose ($P=0.008$) and at 2 h ($P=0.046$). Regression analysis revealed a significant direct association between BMI and fasting glucose ($P=0.002$; $R=0.169$). On simple scatter plot analysis, an inverse correlation was evident between age and fasting plasma glucose level, although this was not significant on regression analysis.
Discussion
This study highlights the variation in glucose tolerance between different ethnicities. It also substantiates the known relationship between BMI and fasting plasma glucose. Historically increasing age has been associated with insulin resistance and is a known risk factor for GDM. Interestingly, this study suggests that fasting glucose amongst GDM patients is inversely related to age, perhaps owing to lifestyle factors and differing perceptions amongst younger patients.

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P363

High prevalence of elevated liver transaminases among 38 727 patients in a diabetes centre in the United Arab Emirates

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Introduction
Liver transaminases are markers of parenchymal liver disease, most commonly non-alcoholic steatohepatitis (NASH), the inflammatory component of non-alcoholic fatty liver disease (NAFLD). Common causes include obesity and type 2 diabetes mellitus (T2DM), both common conditions in the UAE. The prevalence of NAFLD in this population is not known. We report data from a large diabetes centre including ALT and aspartate transaminase (AST) levels in patients with and without diabetes.
Methods
ICLDC adult patient database ($n=38\,727$) was accessed to obtain information on liver transaminase (ALT or AST). These are normally requested as part of the investigation of patients with diabetes mellitus, hyperlipidaemia and other conditions. Patients with AST or ALT over five times the upper limit of normal were excluded from the analysis ($n=99$). Other relevant parameters on patients'

last medical consultation were also analysed.
Results
38 727 out of a total of 61 184 patients (normal glucose tolerance in 13.7%, T2DM in 41.8% and T1DM in 2.1%) over the age of 18 years had liver transaminase levels available. Elevated ALT and AST were noted in 20.0% ($n=7771$) and 12.0% ($n=4643$) of patients respectively. Both enzymes were elevated in 9.66% ($n=3752$) of patients. Mean ALT and AST were 25.5 ± 17.1 and 22.6 ± 9.9 U/l respectively with no statistically significant difference in different weight groups and glucose tolerance categories. There was no significant correlation between waist to hip ratio and ALT or AST in patients regardless of glucose tolerance status.
Conclusions
Elevated liver transaminases are common in the population studied. Weight, abdominal obesity or glucose tolerance status did not appear to be important determinants of this rise. This suggests that conventional markers of body habitus, which are used in European populations, are less valid in the UAE as predictors of the presence of fatty liver.

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P364

Heterozygous deficiency of endoglin decreases insulin and hepatic triglyceride levels during high fat diet

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Introduction
Endoglin is a transmembrane auxiliary receptor for transforming growth factor-β (TGF-β) that is predominantly expressed on proliferating endothelial cells. It plays a wide range of physiological roles but its importance on energy balance or insulin sensitivity has been unexplored.
Material and methods
Heterozygous deficient mice (HZ) were fed standard chow or high fat diet during 16 weeks. Blood glucose levels were measured after an i.p. injection of either D-glucose or insulin. Western blots were performed to measure the protein levels. Liver triglyceride levels was measured using colorimetric methods.
Results
Here we report for first time that heterozygous endoglin deficiency in mice decreases high fat diet-induced hepatic triglyceride content and insulin levels these effects are independent of changes in body weight or adiposity. At molecular level, we failed to detect relevant changes in the insulin signalling pathway.We found decreased triglyceride content in the liver of endoglin heterozygous mice fed a high fat diet.
Conclusion
Our findings indicate that endoglin is a potentially important physiological mediator of insulin levels and hepatic lipid metabolism.

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P365

Prediabetes in human immunodeficiency virus-infected patients: prevalence and clinical significance

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Introduction
Although the importance of disorders in glucose metabolism is well recognized in human immunodeficiency virus (HIV) infected patients; the clinical relevance of prediabetes in this population has been little studied. The aims of this observational study were to determine the prevalence of prediabetes in a cohort of HIV-infected patients on long term highly active antiretroviral therapy (HAART) and to assess whether this condition involves the appearance of particular clinical and metabolic features.
Methods/design
One hundred and five HIV-positive individuals (85.7% men, mean age: 46 ± 6.5 years) were enrolled. Prediabetes was defined using established ADA criteria. Data related to HIV infection, HCV co-infection and anthropometric and metabolic parameters were recorded. Fasting plasma glucose (FPG), fasting insulin levels and homeostatic model assessment-insulin resistance (HOMA-IR) were determined.
Results
Prevalence of prediabetes was higher (43.1%) among HCV/HIV as compared to 38.3% among those with HIV alone. When compared to normoglycemic controls, patients with prediabetes had significantly ($P<0.05$) higher waist-to-hip-ratio (0.96 ± 0.1 vs 0.91 ± 0.1); higher FPG levels (99.4 ± 9.9 vs 90.4 ± 9.6 mg/dl), higher basal insulin (14.1 ± 11.5 vs 8.6 ± 5.1 μIU/l) and higher HOMA-IR (4.4 ± 0.9 vs 1.9 ± 1.2). HCV/HIV with prediabetes were mostly men (100 vs 61%; $P=0.001$) and had significantly ($P<0.001$) lower LDLc levels (113.2 ± 27.2 vs 139.9 ± 37.7 mg/dl) and lower total cholesterol ($180.41\ \pm34.7$ vs 213.89 ± 44.6 mg/dl) as compared to prediabetic HIV group. Although FPG levels and HOMA-IR were higher in prediabetic HCV/HIV patients, the difference was not significantly.
Conclusions
HIV patients on long-term HAART are at risk to develop prediabetes, especially if abdominal obesity is present thus, measurements of HOMA-index and waist-to-hip ratio should be routinely done. The worst lipidic profile in prediabetic HIV alone suggests that this parameter deserves special attention in these individuals.

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P366

Clinical experience of a monogenic diabetes unit during 2008–2012 in the Department of Endocrinology and Nutrition (Málaga, Spain)

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Objectives
MODY diabetes is the most common form of monogenic diabetes, encompassing a heterogeneous group of disorders whose primary defect results from mutations in one of at least seven genes recognized, associated with a primary defect in insulin secretion. It has early onset and autosomal dominant inheritance.
Our objective was to evaluate the results of monogenic diabetes clinic of the Endocrinology and Nutrition Department during 2008–2012.
Methods
After evaluating 103 patients referred to the unit for genotyping, only 75 individuals (from 46 families) were included (34 men and 41 women) using the clinical criteria for the diagnosis of monogenic diabetes as 'Best practice guidelines for the molecular genetic diagnosis of MODY' (Ellard S *et al. Diabetologia* 2008). HNF1A was sequenced in 15 patients (20%), HNF1A/HNF4A in 9 patients (12%), GCK in 38 patients (50.6%), HNF1A/HNF4A/GCK (16%) in 12 patients and HNF1A/HNF4A/GCK/HNF1B in one patient. (1.3%).
DNA extraction was performed from peripheral blood by kit 'MaxwellÒ 16 DNA Purification Kits' (Promega Corporation).
Amplification was performed using 18 pairs of specific primers covering the entire gene GKC and HNF1alfa, and 23 pairs of primers for the gene HNF3A.
The amplification were sequenced in both directions of the PCR products using the ABI automated sequencer from Applied Biosystems 3130.
Subsequent study of the sequences was performed by applying SeqScape Applied Biosystem v2.0 and check Human Gene Mutation Datebase.
Results
Mutations were found in 6 patients HNF1A of 3 families (L12F, A174V, R200W) in 22 patients GCK of 9 families (R191W, G227D, T206M, T209M, R43S, L45P, S453L) and in 3 subject HNF4A (T139I, R331 C) belonging to two families. In 24 subjects were found insulin-resistance polymorphism and 20 patients awaiting results.
Conclusions
The low frequency of mutations suggests the involvement of other genes, not identified in the etiology of MODY, not knowing the influence that could have the presence of genetic variants in other genes related to insulin action about its clinical expression.

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P367

Progression to impaired glucose metabolism in normal glucose tolerant urban population

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Objective
To determine the progression rate to impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes (DM2) and risk factors of progression to diabetes in normal glucose tolerant (NGT) people during 8 years follow up study using WHO 1999 criteria and new criteria of IFG (IFG 5.6-fasting glucose 5.6–6.9 mmol/l).
Research design and methods
This is an 8 year prospective observation in a randomly selected urban population aged $\geq$40 years living in Krakow, Poland. 1752 persons had NGT.based on WHO 1999 criteria. 564 of invited person (209 men and 355 women, aged mean 60.7, S.D.$=$9.2) attended the follow-up assessment. Subjects underwent a physical examination including weight/height, waist circumference, biochemical examination including OGTT glucose/insulin and questionnaire examination concerning CVD health history and family history of type 2 diabetes. Multiple logistic regression was used to assess the risk factors of progression to diabetes.
Results
The prevalence of DM2, IFG and IGT according to WHO 1999 criteria in examined population with baseline NGT was 4.43, 3.37 and 9.93% respectively. The prevalence of IFG (IFG 5.6) using new criteria, was 13.48%. Lowering cutoff point for IFG caused 10.11% increase in the prevalence of IFG. Risk factors of the conversion to diabetes in NGT WHO 1999 were age, 120'OGTT glucose, insulin and hyperinsulinaemia. Risk factors of the convertion to diabetes in NGT 5.6 mmol/l were 120'OGTT glucose. Risk factors of the convertion to diabetes in IGT WHO 1999 were 120'OGTT glucose.
Conclusion
In the studied baseline NGT population after 8 years of follow up high progression rate to impaired glucose metabolism was found. The implementation of new IFG diagnostic criteria increased the prevalence of IFG by 10.1%. Fasting glucose and OGTT glucose should be measured for early identification of people with impaired glucose metabolism.

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P368

Impact of the insulin pump therapy on quality of life children and adolescents with diabetes mellitus type 1

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Objectives
To investigate the changes parameters of quality of life (QoL) in children and adolescents with diabetes mellitus type 1 (T1DM), using different regimes insulin therapy.
Research design and methods
Thirty patients with diabetes mellitus type 1, aged 5–18 years (mean 13.3$\pm$3.04 years), with disease duration from 1 to 10 years (mean 5.4$\pm$3.4 years) took part in paired study. QoL was assessed with the PedsQLä (Generic Core Scales and Diabetes Module, Russian version) at two time points: 1st – during multiple daily insulin injection (MDI); 2nd – after initiation continuous subcutaneous insulin infusion (CSII). Data were analyzed in subgroups aged 5–7, 8–12, and 13–18 years.
Results
Nevertheless, analysis demonstrated positive dynamic of QoL in patients after initiation CSII. Treatment barriers decreased due to reduction of a number of injections (75.3 vs 90.0 $P<0.05$) in preschool-age children. Patients at the age of 8–12 evaluated higher physical function (74.2 vs 94.0, $P<0.05$) after initiation CSII. In adolescent's group social activity indicates improved (85.0 vs 95.0, $P<0.05$). Further, they were less worried about long-term complications (70.0 vs 85.0, $P<0.05$).
Conclusions
Using of insulin pump therapy in children with T1DM allows to improve of quality of life. Preschoolers get used to the necessary of insulin therapy easier, schoolchildren and adolescents have opportunity to increase the level of their physical and social activity.

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P369

Relation of serum chemerin level with markers of atherosclerosis in diabetes and prediabetes

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Introduction
Chemerin is a novel adipokine that is correlated with adipocyte differentiation, glucose metabolism and inflammation, but its role remains to be elucidated in atherosclerosis particularly in diabetes and prediabetes. We aimed to investigate the relation of serum chemerin levels with markers of atherosclerosis as exemplified by pulse wave velocity (PWV), carotid intima media thickness (CIMT), and carotid plaque presence in these groups.
Methods and materials
We included age, BMI, and gender matched 30 patients with type 2 DM, 19 patients with prediabetes, and 25 healthy individuals with normal glucose tolerance. Serum chemerin levels, lipid parameters, fasting glucose and insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR) were assessed. PWV, CIMT, anthropometric measures, body fat percent, and epicardial fat thickness were recorded.
Results
Serum chemerin levels were similar among groups (226.5$\pm$36.8 ng/ml in controls, 242.5$\pm$42.8 in prediabetes, 233.0$\pm$40.4 in diabetes, $P=0.338$), though glucose metabolism parameters including HOMA-IR were higher in patients with type 2 DM. PWV was higher in diabetes than in prediabetes and controls ($P=0.039$). Chemerin levels were positively correlated with BMI ($P<0.001$, $r=0.425$), total cholesterol ($P=0.042$, $r=0.239$), triglyceride ($P=0.038$, $r=0.246$), body fat percent ($P=0.007$, $r=0.313$) and left CIMT ($P=0.032$, $r=0.249$) in whole group. BMI and body fat percent were also correlated with chemerin in diabetic group whereas body fat percent and left CIMT remained to be correlated with chemerin in prediabetic group. Eleven patients in the diabetic group, two patients in the prediabetic group and three individuals in the control group had carotid plaque. Chemerin levels were comparable in patients with diabetes when grouped according to the presence of carotis plaque.
Conclusion
Although chemerin is not correlated with atherosclerosis markers as measured by PWV and carotid plaque in diabetes and prediabetes, positive correlation with body fat percent in total group and positive correlation of CIMT and chemerin in prediabetes still arise an unanswered question about its role in atherosclerosis requiring further investigations.

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P370

Serum vaspin levels in women with and without gestational diabetes mellitus during pregnancy and postpartum

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Although vaspin is regarded an insulin-sensitizing adipokine, its role in gestational diabetes mellitus (GDM) is currently unknown. We aimed to evaluate serum vaspin levels and their correlation with insulin resistance in women with and without GDM. Forty-four women with GDM (GDM Group 20 managed with diet only (GDM-diet) and 24 with diet plus insulin (GDM-insulin)) and 44 age-matched pregnant women with normal glucose tolerance (control group) were studied. Serum glucose, lipids, uric acid, insulin, and vaspin were measured at the 2nd and 3rd trimesters of pregnancy and postpartum. The quantitative insulin sensitivity check index (QUICKI) and homeostasis model of assessment-insulin resistance (HOMA-IR) were calculated. Circulating vaspin levels decreased significantly postpartum in all groups ($P<0.001$), but did not differ between GDM or GDM subgroups and control group in any time point. At the 3rd trimester

of pregnancy vaspin was positively correlated to insulin ($P=0.022$), HOMA-IR ($P=0.016$) and triglycerides ($P=0.033$) and negatively correlated to QUICKI ($P=0.016$) in the GDM women, but not in the Controls. These correlations were not observed at the 2nd trimester or *postpartum*. Vaspin, in contrast to HOMA-IR, could not independently predict GDM in binary logistic regression. In patients with GDM, insulin treatment did not affect vaspin levels. In conclusion, our data suggest that vaspin levels gradually decrease from the 2nd trimester to postpartum; however, decreases are similar between women with or without GDM. Serum vaspin cannot independently predict GDM and it is not affected by the degree of glucose metabolism deregulation or the exogenous administration of insulin.

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P371

Abstract withdrawn.

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P372

Association between toll-like receptor 4 polymorphism and type 2 diabetes mellitus

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Introduction
Type two diabetes (T2DM), is the most common form of diabetes and known to be associated with low-grade inflammation. Since TLR-4 plays an important role in bacteriel recognition of lipopolysaccharide (LPS) and activation of proinflammatory pathways we aimed of to test whether TLR-4 gene polymorphism plays a protective role in the development of T2DM in a pilot Turkish population.
Description of methods/design
Blood samples were collected from 58 patients with T2DM and 63 healthy people. Allelic variants of TLR-4 (Asp299Gly and Thr399Ile) were assayed by real-time PCR. Genomic DNA was amplified using FAM/VIC primers spesific for allelic variants of TLR-4 Asp299Gly (refSNP ID: rs4986790) and Thr399Ile (refSNP ID: rs4986791) with real-time PCR. Amplicons were analyzed with high resolution melting at Light Cycler 480 for detecting different melting patterns of polymorphic and wild type alleles.
Results and conclusion
For genotype 299 gen polymorphism; 17.2% of the patients ($n=10$) with T2DM were heterozygotic (Asp299Gly), 6.8% ($n=4$) of them were homozygotic (Gly299Gly) mutant. 9.5% ($n=6$) of the controls were heterozygotic and 6.3% ($n=4$) of them were homozygotic. Heterozygotic and homozygotic mutation between patients with T2DM and controls were similar ($P=0.21$, $P=0.9$ respectively)For genotype 399 gen polymorphism; 8.6% of the patients ($n=5$) with T2DM and 9.5% ($n=4$) of the controls were heterozygotic (Thr299Ile). None of them were homozygotic (Ile299Ile) mutant. Heterozygotic mutation between patients with T2DM and controls were similar ($P=0.63$). The cumulative mutations number of the subjects with TLR-4 gene polymorphism were. similar in groups (17 vs 12, $P=0.18$). Our results demonstrated that TLR-4 gene polymorphism has no association with T2DM.

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P373

Altered reactivity of pituitary–adrenal axis to stimulation tests and altered tissue metabolism of cortisol in long-standing type 1 Diabetes

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This work aimed to evaluate pituitary–adrenal axis and tissue metabolism of cortisol in patients with long standing type one diabetes.
Fifty patients with diabetes type 1 were investigated; age 38 ± 10 years (mean $\pm$ S.D.), age at diagnosis 25.5 ± 10 years, disease duration 15 ± 8 years, BMI 24.5 ± 2.7 kg/cm^2, HbA1c 7.2 ± 1.2%. Control group consisted of 40 healthy volunteers.
The study was approved by the Ethical Committee.
Adrenocortical function had been tested by three different tests – Synacthen test, CRH test, and peripheral metabolism of cortisol had been evaluated by cortisone acetate (25 mg) administration and analysis of cortisol:cortisone ratio.
We evaluated serum ACTH, serum cortisol, serum cortisone during these tests, at the basal level cortisol binding globulin, aldosterone, and metabolic parameters of diabetics. Patients with positivity of adrenal autoantibodies, pituitary autoantibodies, thyroid disorders were excluded from group of these patients.
In 15% of patients we have found a subnormal response (<500 nmol/l) of the serum cortisol during low-dose Synacthen test, accompanied by significantly decreased stimulated values of aldosterone. Basal serum cortisol, aldosterone has been significantly reduced, while ACTH, cortisol binding globulin did not change. The CRH test displayed the low response in serum cortisol and hyperactivity in ACTH in this group of patients. The course of cortisol after cortisone acetate administration was delayed and significantly different in diabetic patients compare to controls.
Conclusion
At least in a part of patients with type 1 diabetes mellitus a reduced secretion of both cortisol and aldosterone after ACTH stimulation could be observed. In diabetic patients peripheral conversion cortisol-cortisone is disturbed as well. These results may contribute to better understanding to pituitary–adrenal adaptation in diabetes type one.

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P374

The effects of gestational diabetes on basal metabolic rate in pregnancy

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Introduction
The total energy expenditure in pregnancy is increased largely due to elevated basal metabolic rate (BMR) during pregnancy. The aim of this study is to evaluate the effects of gestational diabetes on basal metabolic rate, body weight and HbAlc, by comparing these parameters in gestational diabetic and healthy pregnant women.
Method
Fifty-eight gestational diabetic women (GDM) and fourty healthy non diabetic pregnants who had normal thyroid function and not taking any medication which may possibly alter the metabolic rate, recruited in the study. Mean ages of the study population were 21–42 years. GDM was diagnosed by a 100 g-OGTT after 50 g oral glucose challenge test. Basal metabolic rate was measured by oxygen consumption after 8 h overnight fasting, also BMI, fasting blood glucose (FBG) and HbA1C were measured in gestational weeks of 24–32. Due to body weight gain as expected during pregnancy, BMR was divided by BMI to ensure normalization.
Results
The rate of BMR to BMI was found 50.30 ± 10.58 kcal/day in GDM group and 51.98 ± 9.70 kcal/day in non-GDM group. The difference was not statistically significant. FBG and HbA1c were higher in GDM women as expected. No statistically significant relationship was found either between BMR and FBG and HbA1c levels and body weight.
Discussion
In our study, the average BMR was not different in GDM women compared to pregnants who do not have gestational diabetics. These results might be different in the third trimester of pregnancy instead of second trimester.

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P375

Gene expression of TLR2, TLR4, and TLR9 in human PBMCs with diabetes

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Objective
To investigate the relationship between expression of TLR2, TLR4, and TLR9 in PBMCs and type 2 diabetes and their significance in pathogenesis of diabetes.
Methods
Expression of TLR2, TLR4, and TLR9 in PMBCs was measured by flow cytometry in 112 subjects with coronary heart disease (CHD, $n=49$), type 2 diabetes plus coronary heart disease (DM+CHD, $n=34$), type 2 diabetes (DM, $n=4$) and healthy controls (CONTROL, $n=25$) were analyzed. The results were calculated by MFI and MPP respectively.
Results
There was no significant difference of expression of TLR2, TLR4, and TLR9 in PMBCs among study groups. The positive rate or MFI of TLR2 in monocyte was significantly higher than that in lymphocyte. There was no significant difference of expression of TLR4 and TLR9 between monocyte and lymphocyte respectively.
Conclusions
There is no significant relationship between the expression of TLR2, TLR4, TLR9 in PMBCs and diabetes. TLR2 is mainly expressed in human monocytes. TLR4 and TLR9 are expressed in both human monocytes and lymphocytes.
Keywords: Type 2; Diabetes; Coronary heart disease; Toll-like receptors; flow cytometry
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P376

Prevalence of achievement of HbA1c, blood pressure, and cholesterol (ABC) Goal in Koreans with diabetes

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Introduction
Diabetes is one of the most challenging health problems in the world. The degree of prevention or control of complications directly affects patients' mortality. Therefore, it is crucial to control the risk factors of diabetes. We investigated the prevalence, treatment, and control of diagnosed diabetes in Korean adults from 1998 to 2010.
Methods
The Korean Ministry of Health and Welfare conducted the Korean National Health and Nutrition Examination Survey (KNHANES) in the years 1998 (I), 2001 (II), 2005 (III), 2007–2009 (IV), and 2010 (V). We estimated the prevalence of diagnosed diabetes in Korean adults and the proportions of well-controlled diabetes, as defined having HbA1c $<7.0\%$, blood pressure $<130/80$ mmHg, and LDL cholesterol <100 mg/dl.
Results
The prevalence of diagnosed diabetes increased significantly from 3.2% in 1998 to 6.4% in 2010 ($P<0.0001$). The prevalence of adults with diagnosed diabetes achieving blood pressure, and LDL cholesterol target levels increased from 23.8% to 54.2% ($P<0.0001$), and 25.7% to 47.7% ($P<0.0001$) respectively. But the percentage of achieving glycemic goals did not increased significantly from 42.5 to 49.1% ($P=0.3034$). Furthermore, there were significant increases in the proportions of individuals achieving all three target levels, from 2.7% in 2005 to 8.7% in 2010 ($P<0.0001$).
Conclusion
The prevalence of diagnosed diabetes in Korea increased significantly from 1998 to 2010. The percentages of those achieving all recommendations of controlling risk factors have increased, but are still not satisfactory.
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P377

Nutritional status assessment in patients with cystic fibrosis

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Introduction
Cystic fibrosis (CF) is a chronic disease at risk of malnutrition. The aim of the study is to assess nutritional status of patients with CF.
Methods
Descriptive study of CF patients referred for a nutritional evaluation. Data collected included lung function, pancreatic and carbohydrate metabolism (according to ADA's diagnostic criteria), anthropometric and laboratory parameters. Results are shown as mean (S.D.).
Results
Sixty four patients were studied (56.3% males) with a mean age of 26.8 (7.4) years.100% of them had respiratory involvement and 93.4% exocrine pancreatic disfunction. 29.5% underwent lung transplantation, with a mean age of 22.4 (1.7) years. 50% of patients were diabetic and 21.9%, prediabetic. 37.5% received systemic treatment with corticosteroids. BMI was 19.2 (2.7) kg/m^2. The ideal percentage weight was 83.9% (13.3) and weight loss in the last 6 months was 3.6% (5.8). The main causes of weight loss were respiratory infections (55.6%), decreased appetite or early satiety (32.1%), and steatorrhea (14.8%). Vitamin D deficiency was present in 60.9%, vitamin A in 70.6% and vitamin E in 72.5%. Severe malnutrition was diagnosed in 20.4% of patients, moderate in 22.2% and mild in 25.9% of cases. Mixed malnutrition was found in 51.4%, protein in 29.7% and caloric in18, 9%. Diabetes was associated with vitamin D deficiency <30 ng/ml ($P=0.01$) and lung transplantation ($P<0.001$). Severe malnutrition is statistically associated with lung transplantation ($P=0.01$), systemic corticosteroid therapy ($P=0.01$), and diabetes ($P=0.04$).
Conclusion
A high prevalence of malnutrition (73%) was found in CF patients. Severe malnutrition was associated with lung transplantation, systemic corticosteroid therapy and diabetes. Pretransplant assessment of nutritional status is important in CF patients. Diabetes was associated with lung transplantation and vitamin D deficiency.
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P378

The expression mechanism of lipocalin-2 by co-stimulation with interleukin 1β and interferon γ in RINm5F β-cells

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Lipocalin-2 (LCN-2) is known to act as an antiinflammatory or a proinflammatory mediator. Recently, LCN-2 has been recognized to play a role in obesity and insulin resistance. However, there is no knowledge about the expression and the role of LCN-2 in pancreatic islet β-cells. Therefore, we examined molecular mechanisms by which proinflammatory cytokines interleukin 1β (IL1β) and interferon γ (IFNγ) induce LCN-2 expression in RINm5F β-cells. IL1β significantly induced LCN-2 protein and mRNA expression. INFγ alone did not induce LCN-2 protein and mRNA expression whereas IFNγ significantly stimulated IL1β-induced LCN-2 expression. However, promoter study and EMSA showed that INFγ failed to stimulate IL1β-induced LCN-2 promoter activity and binding activity of NFκB on LCN-2 promoter. Meanwhile, western blot using NFκB inhibitor and promoter assay using truncated constructs showed that NFκB was a key factor in IL1β-induced LCN-2 expression. However, NFκB and STAT-1 were not involved in stimulatory effect of INFγ on IL1β-induced LCN-2 expression. Furthermore, we found that LCN-2 expression was significantly increased and prolonged compared with both iNOS and COX-2 expression under exposure to IL1β, and that LCN-2 receptor was expressed in pancreatic β-cells. Our data suggest that IL1β induced LCN-2 expression via NFκB activation and IFNγ significantly stimulated IL1β-induced LCN-2 expression at protein and mRNA level but not at promoter activity. This effect of INFγ was not independent of NFκB and STAT-1 activation. In addition, abundant expression of LCN-2 and LCN-2 receptor in β-cells implies that LCN-2 plays a role in β-cell function. Our data suggest that IFN-γ significantly potentiates IL1β-induced LCN-2 expression at mRNA and protein level but not at promoter level, and NFκB plays a key role in IL1β-induced LCN-2 expression. In

addition, abundant expression of LCN-2 and LCN-2 receptor in β-cells implies that LCN-2 plays a role in β-cell function.

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P379

The effect of CCR2 G190A gene polymorphism on development of diabetic coronary artery disese

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Introduction
Inflammatory mechanisms play an important role in the development of cardiovascular diseases and diabetes. Chemokines are chemotactic cytokines that orchestrate leukocyte trafficking in tissues, thus, playing an important role in regulation of immunological processes. Some studies have shown that CCR2 G190A polymorphism might be important for protection of susceptibility to some immunologically-mediated disorders. The aim of our study was to investigate the effect of the CCR2 G190A polymorphism on the development of diabetic coronary heart disease.
Methods
Sixty two diabetic patient with coronary heart disease, 81 diabetic patient without coronary heart disease, and 62 disease-free controls were genotyped. Diabetes was diagnosed according to the criteria of the American Diabetes Association (ADA) and the nondiabetic subjects were selected according to no past history of diabetes mellitus or impaired glucose tolerance. The CCR2 G190A genotype was determined by PCR followed by RFLP.
Results
No significant difference in the frequency of the CCR2 polymorphism between patient groups and the controls was found (P : 0.78 X^2 0.48).
Conclusion
In contrast, we did not find any significant difference in CCR2 G190A polymorphism in diabetic patients with coronary heart disease, diabetic patient without coronary heart disease and healty controls

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P380

High prevalence of the metabolic syndrome among newly diagnosed type 2 diabetes patients in Denmark

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Few population-based data exist on the prevalence of the metabolic syndrome (MS) its components, and its association with subclinical inflammation and insulin resistance among patients newly diagnosed with type two diabetes (T2D). We examined the MS according to IDF criteria by linkage primary data (waist circumference, fasting blood glucose, and high-sensitivity C-reactive protein (CRP)) with variables from the Danish diabetes database for adults (blood pressure and lipids) and a nationwide prescription database (antilipidemic, antihypertensive, and hypoglycemic drugs).
We included 525 newly diagnosed T2D patients (median age 60 years, 60% men), whereof 89.0% had MS. Age and gender were similar in MS and non-MS patients. Waist circumference was elevated in 98.1% of MS vs 34.5% of non-MS patients (prevalence ratio 2.84; 95% CI: 2.49–3.20). Most MS patients had elevated blood pressure (systolic $\geq$130, or diastolic $\geq$85, or antihypertensive drug): 94.4 vs 55.2% in non-MS patients (prevalence ratio 1.71; 95% CI: 1.48–1.94). Fasting blood glucose was increased in nearly all MS and non-MS patients. Triglycerides were elevated in 48.6% of MS and 3.4% of non-MS patients (prevalence ratio 14.10; 95% CI: 12.73–15.46). HDL-cholesterol was reduced in 33.6% of MS and 0.0% of non-MS patients. Increased C-peptide levels $\geq$300 pmol/l was more common in MS than non-MS patients (96.8 vs 77.6%, $P=0.0005$). 23.1% of the MS patients were previous hospital-diagnosed with cardiovascular disease vs 0% in non-MS. The median CRP was 2.40 mg/l (quartiles 1.00–5.10) in MS patients vs 1.20 mg/l (quartiles 0.60–3.50) in non-MS patients ($P=0.006$).
89% of the newly diagnosed T2D patients have the MS, with 90% having elevated waist circumference, blood pressure and blood glucose, and <50% having elevated triglycerides and reduced HDL-cholesterol. Elevated C-peptide levels are common in T2D patients with MS and their CRP level and risk of previous cardiovascular disease is higher than in non-MS patients.

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P381

Trans-operative and post-operative medical complications in patients with diabetes mellitus type 2

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Purpose
To determine the association between clinical indicators of metabolic state and cardiovascular, infectious and metabolic complications during or after elective surgery in patients with type 2 diabetes mellitus (T2DM) regardless of their treatment scheme.
Methods
Prospective observational study. Patients with T2DM, ages 20–80 years, scheduled for elective surgery were evaluated with office BP, BMI, Hb1Ac, fasting glucose, fibrinogen, total cholesterol, triglycerides, ischemic cardiomyopathy by EKG, and the presence of LVH by echocardiography. Cardiovascular, infectious and metabolic complications during or after elective surgery were registered till the time of discharge. Patients with cetonuria, hypoglycemia or clinical conditions that could interfere with the diagnosis of persistent microalbuminuria and autonomic neuropathy were excluded. Significant associations were determined through chi square tests and linear regression analyses.
Results
Sixty two patients were evaluated (mean age: 60.5 years, 20–80 years range; 69.6% females). The most frequent complications were hypertension ($n=21$), hyperglycemia ($n=24$) and surgical wound infection ($n=20$). Analysis identified a significant association between age ($P=0.04$; OR: 4.98, S.E.M.: 0.73 (B: 1.60, $P=0.02$)), Hb1Ac $\geq$7% ($P=0.01$; OR: 12.77, S.E.M.: 0.92 (B: 2.54, $P=0.006$)), EKG with ischemic cardiomyopathy ($P=0.04$; OR: 4.14, S.E.M.: 0.82 (B: 1.42, $P=0.08$)) and general anesthesia ($P=0.03$; OR: 10.63, S.E.M.: 0.83 (B: 2.36, $P=0.004$)) with more cardiovascular complications during or after surgery; glucemia $\geq$126 mg/dl ($P=0.04$; OR: 3.62, S.E.M.: 0.74 (B: 1.28, $P=0.08$)) and Hb1Ac $\geq$7% ($P=0.01$; OR: 6.43, S.E.M.: 0.81 (B: 1.84, $P=0.02$)) were associated with more metabolic complications during or after surgery; No other parameter measured was significantly associates with cardiovascular, infectious or metabolic complication during or after elective surgery.
Conclusions
Significant association were found between age, Hb1Ac levels, type of anesthesia and EKG with evidence of ischemic cardiomyopathy and cardiovascular complications; preoperative glycaemia and Hb1Ac were associated with metabolic complications. The evaluation of the metabolic status of T2DM patients before elective surgery may help to anticipate trans- or post-operative complications.

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P382

Assessment of pre-pregnancy BMI in patients with gestational diabetes

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Objective
Relationship between pre-pregnancy BMI (PPBMI) in gestational diabetic (GD) patients, and age, country of origin, family history of type 2 diabetes mellitus (T2DM), GD history, and BMI after pregnancy.
Methods
Transversal, descriptive study of 259 consecutive pregnant with confirmed DG. Analysis were based on Fisher's exact test and Student's t-test. A $P<0.05$ was significant (SPSS 19.0).

Results
Mean age was 34.3 years, 49.8% primiparous, 66.7% Spanish, 17.4% South American, 5.5% Eastern European, 3.1% North African, 2.0% South Africans, 3.5% Chinese, 0.8% Indian, and 0.4% Portuguese/Cuban. Women with a family history of T2DM had a mean average of 2.51 kg/m^2 PPBMI higher than pregnant without such a history ($P<0.001$). The mean PPBMI was 27.1 kg/m^2 (30% overweight and 28.0% obese). Thirty-three percent of Spanish pregnant were overweight and 25.4% obese, vs 22.6 and 33.3% respectively amongst the other countries. A positive correlation was observed between age and PPBMI ($P=0.012$). There was a statistically significant difference for the PPBMI of the primiparous compared to the multiparous, with a mean difference of 2.4 kg/m^2 in favour of the latter. *Postpartum* mean BMI between, 4 and 6 months after delivery was 27.2 kg/m^2 showing no significant difference to the PPBMI.
Conclusions
Is interesting the high average age of the pregnant, the high percentage of overweight and obese women before pregnancy and the positive correlation of PPBMI and age in all pregnant. However PPBMI is higher in multiparous and was positively correlated with family history of T2DM. The data observed in the study population suggest the possibility of an increase in the development of T2DM in these patients, so it will be advisable to tighter control of the same.

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P383

Study of the development and validation of the specific "Satiglu" questionnaire to evaluate the satisfaction of diabetic patients with glucometers

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Objective
To develop a specific questionnaire to evaluate the satisfaction of diabetic patients with the use of glucometers and evaluate their psychometric properties (feasibility, reliability, and validity).
Material and methods
Two stages: i) questionnaire development: a set of 53 items was constructed after a bibliographic review. A panel of six experts and 50 patients concluded a 25-item version of the questionnaire (dimensions: satisfaction, inconvenience, added value, and appearance). ii) Questionnaire validation: 6-month, epidemiological, multicentre, cross-sectional study that recruited 286 adult patients with type 1 or 2 diabetes mellitus (DM) who used a glucometer. Their responses to the questionnaire were collected together with supplementary variables: sociodemographic and clinical, adherence to treatment (Morisky-Green), generic satisfaction with treatment questionnaire, SAT-Q, and also specifically for diabetes, DTQS-s. The questionnaire's test-retest reliability was evaluated by 51 patients completing the questionnaire 15 days after the first visit.
Results
The mean age (SD) of the sample was 42.70 years (16.25), 55.30% were women and 63.70% presented type I DM. The patients' mean percentage (S.D.) of HbA1c was 7.82% (1.45). Mean adherence was 7.65 (1.66), the patients had being using a glucometer for a mean time of 6 years, and average glycaemia controls were 3.95 (1.76). 73.50% of the patients had obtained the glucometer from an endocrinologist.
Reliability
Cronbach's alpha was 0.88 and ranged from 0.61 to 0.82 for the different subscales. The intra-class coefficient of correlation for the total SATIGLU score was 0.75.
Validity
The SATIGLU questionnaire showed a positive, statistically significant correlation with SAT-Q and DTQ9-s scores and the patients' degree of adherence to treatment.
Conclusions
The SATIGLU questionnaire shows appropriate psychometric properties as an instrument to evaluate patient satisfaction with the use of glucometers.

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P384

Inflammatory markers in diabetic foot and impact of vitamin D deficiency

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Objective
i) To evaluate plasma levels of IL6, adiponectin and resistin in subjects with diabetic foot in comparison with subjects without foot complications. ii) To assess the impact of vitamin D status on the levels of above inflammatory markers.
Methods
A total 100 diabetic foot cases and 100 diabetic controls were recruited in the study. Serum level of 25OH vitamin D was estimated from the cases and controls by RIA. Serum IL6, adiponectin and resistin were assayed by ELISA. Data were analyzed using online graphpad Quickcalc Software and $P<0.05$ was considered statistically significant.
Results
Mean age of the study population was 54.3 ± 12.4 years (male:female = 68:32). Mean age of the controls was 52.5 ± 13.6 (male:female = 60:40). HbA1c was comparable (10.3 vs 10.9%). Diabetic foot cases were having lower vitamin D status (16.1 ± 16.0 ng/ml) than the diabetic controls (19.8 ± 14.1 ng/ml). Prevalance of vitamin D deficiency was higher in cases than controls (62 vs 57%). Females outnumbered males in terms of prevalence of vitamin D deficiency (22/32 females (68%) vs 40/68 (58%) males in cases and 25/ 40 females (62%) vs 32/60 (53%) males in control group).Severity of vitamin D deficiency (<10 ng/ml) was higher in cases (48.2%) than controls (26.2%).
IL6 level was higher in cases (128.3 pg/ml) than the controls (63.8 pg/ml) ($P-0.01$). Similarly lower median plasma levels of adiponectin (7.7 vs 8.4 µg/ml) and higher median plasma levels of resistin (3.8 vs 3.6 ng/ml) were observed in cases ($P<0.05$). No significant difference was observed in the levels of these markers between male and female study participants in both the groups. Patients under vitamin D deficient group (<30 ng/ml) demonstrated higher IL6 (130.8 vs 100.0 pg/ml), higher resistin (3.9 vs 3.6 ng/ml) and lower adiponectin (7.6 vs 8.3 µg/ml) levels compared to vitamin D sufficient (≥30 ng/ml) group in diabetic foot ($P<0.05$).
Discussion
Immuno-regulatory role of vitamin D is well established. Diabetic foot infections reflect the immune-compromised state of the patients and therefore it is speculative that vitamin D deficiency is more common and severe in diabetic foot. Our study demonstrated that diabetic subjects with diabetic foot showed in comparison with diabetics without diabetic foot higher IL6 and resistin plasma levels and lower adiponectin plasma levels. Hypovitaminosis D is more prevalent in patients with diabetic foot and vitamin D deficiency is more severe in patients with diabetic foot infections. The levels of the above markers are more in diabetic foot patients with vitamin D deficiency.
Conclusion
Assumption is made that vitamin D deficiency enhances inflammatory response in addition to hyperglycemia, in diabetic foot.

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P385

Study of the prevalence of subclinical hypothyroidism in type 2 diabetic Egyptian women

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Background
Subclinical hypothyroidism is a common thyroid disorder. Although there is a recognized association between thyroid disease and diabetes mellitus, there is no definite answer as to whether screening for subclinical hypothyroidism is necessary in patients with type 2 diabetes.
Objective
To evaluate the prevalence of subclinical hypothyroidism in women with type 2 diabetes in Egypt.
Patients and methods
women with type 2 diabetes (54.3 ± 9.3 years) and 100 women without diabetes (49.2 ± 5.3 years) participated. Those with type 1 diabetes, thyroid disorders, chronic illness or receiving drugs that affect thyroid function were excluded. They were assessed for FBS, HbA1c, lipid profile, ultrasensitive CRP, urine

albumin:creatinine ratio and thyroid parameters as TSH, FT_4, anti-thyroid peroxidase antibodies (TPO), and thyroid volume by neck ultrasound.
Results
The prevalence of subclinical hypothyroidism was 9% in diabetic patients and 3% in control subjects with no significant statistical difference between the two groups ($P=0.09$). FT_4 was lower in diabetic group than controls with $P<0.001$. TSH was higher in diabetics than control with $P<0.01$. The prevalence of TPO was similar in both groups ($P=0.3$) as well as the thyroid volume ($P=0.8$). Across all participants, positivity for TPO was significantly higher in patients with subclinical hypothyroidism than in euthyroid subjects. usCRP was higher in diabetic patients ($P<0.001$), and was positively correlated with parameters of the metabolic profile rather than the thyroid profile parameters. In our study, thyroid parameters among different stages of diabetic nephropathy showed no significant difference, but patients with macroalbuminuria have the lowest FT_4 values and highest TSH values.
Conclusion
The statistical insignificant difference in the prevalence of subclinical hypothyroidism in women with type 2 diabetes and women without diabetes suggests that routine screening of thyroid function in patients with type 2 diabetes is unwarranted. However, further mega studies are recommended.

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P386

Maternal serum fetuin-A, leptin, and hs-CRP concentrations in gestational diabetes

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Aim
The aim of this study was to examine serum fetuin-A, leptin and hs-CRP concentrations among pregnant women with gestational diabetes (GDM), and to evaluate the relationship between these parameters and insulin resistance.
Materials and methods
In this study, we evaluated 47 pregnant patients with GDM. As the control group, 43 normal pregnant women having similar properties with these GDM patients, but not gestational diabetes were enrolled. Serum fetuin-A, leptin, and high sensitive CRP (serum reactive protein) levels of both groups of pregnants were measured. Insulin sensitivity and insulin resistance were calculated by QUCKI-IS (quantitative insulin check index) and HOMA-IR (homeostasis model assessment of insulin resistance) methods respectively.
Results
Fasting serum glucose levels ($P<0.001$), fasting serum insulin levels ($P=0.002$), HOMA-IR index ($P=0.001$), serum leptin levels ($P=0.002$), and serum hs-CRP levels ($P<0.001$) of the women with GDM were found statistically significantly higher than the control group. Serum fetuin-A levels and HOMA-β index showed no statistically significant difference between the two groups.
Conclusion
Pregnant women with and without GDM were compared in terms of serum fetuin-A levels and no statistically significant difference was detected between the two groups. In addition, insulin resistance calculated by HOMA-IR and fetuin-A showed no significant correlation between the two groups of patients. Serum leptin and hs-CRP concentrations increased in women with GDM, and a significant positive correlation was found between serum leptin hs-CRP and insulin resistance

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P387

The impact of fasting during Ramadan on the glycemic control of patients with type 2 diabetes mellitus

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Background
Millions of Muslims fast from dawn until dusk during the annual Islamic holy month of Ramadan. Most of the studies evaluating biochemical changes in diabetic patients during Ramadan showed little changes in the glycemic control. In this study, our aim was to assess the impact of fasting during Ramadan on glycemic control in patients with type 2 diabetes.
Methods and design
We examined 122 patients with type 2 diabetes (82 females, 40 males, age 56.93±9.57 years) before and after the Ramadan. 66.4% of the patients were treated with oral antidiabetic (OAD) alone, 7.4% with a combination of OAD plus exenatide, 6.5% with a combination of insulin plus OAD and 19.7% with insulin alone. 88 of 122 patients fasted during Ramadan (26.98±5.93 days). Weight, BMI, waist circumference, blood pressure, fasting plasma glucose (FPG), postprandial glucose (PPG), fructosamine, HbA1c, fasting insulin, and lipid parameters were measured.
Results
The frequencies of both severe hypoglycemia and hyperglycemia were higher in the fasting group, but the difference was not significant ($P=0.18$). Weight (89.86±17.20 vs 89.22±16.68 kg, $P=0.069$), BMI (36.32±15.33 vs 35.71±11.47 kg/m^2, $P=0.30$), waist circumference (106.97±15.57 vs 106.06 ±14.04 cm, $P=0.535$), blood pressure, FPG (143.38±52.04 vs 139.31 ±43.47 mg/dl, $P=0.758$), PPG (213.40±98.56 vs 215.66+109.31 mg/dl, $P=0.634$), fructosamine (314.18±75.40 vs 314.49±68.36 µmol/l, $P=0.114$), HbA1c (6.33±0.98 vs 6.22±0.92%, $P=0.057$), fasting insulin (12.61±8.94 vs 10.51±6.26 µU/ml, $P=0.200$) and lipid parameters were unchanged in patients who fasted during Ramadan.
Conclusions
In this study, we concluded that fasting during Ramadan did not worsen the glycemic control of patients with type 2 diabetes.

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P388

Identification of plasma biomarkers in human diabetic retinopathy

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Retinopathy has been observed in around 25% of patients with type one and type two diabetes for 3–5 years. Diabetic retinopathy can cause poor vision and even blindness since high glucose has been evidenced to weaken retinal capillary leading to leakage of blood into the surrounding space. Therefore, the prevention, diagnosis and therapy of diabetes retinopathy are very important in diabetes patients. Here, we adopted a proteomics-based approach using 2D-DIGE and MALDI-TOF/TOF MS to compare the differential plasma proteome between diabetic retinopathy with significant retinopathy occurrence within 5 years after diagnosis of diabetes, and diabetic non-retinopathy without diagnosed retinopathy for more than 10 years after diagnosis of diabetes. In this study, we identified proteins differential expression in the plasma of diabetic retinopathy patients. These proteins mainly have inflammatory response and coagulation roles, and may be associated with the progression and development of diabetes. In conclusion, we report a comprehensive patient-based plasma proteomic approach to the identification of potential plasma biomarkers for diabetic retinopathy screening and detection.

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Diabetes awareness in the general population of northern Greece

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Introduction
The current study was conducted to assess the level of awareness about diabetes in a random population.
Methods
A questionnaire was conducted by communication over the telephone in Thessaloniki, Macedonia, and Greece in a random population of 600 persons. Presence of DM1 or DM2 in the person questioned or a family member, knowledge of precipitating factors, duration, curability, choice of phycisian and compliance with medication were assessed.
Results
In the total population, 14% had diabetes. 41% reported their first choice of phycisian would be an endocrinologist, 36% an internist, 5% a general phycisian and 18% (DKn/NA). As causal factors 63% reported hereditary predispotition as the primary factor and of secondary importance poor eating habits, obesity, lack of physical activity and environmental pollution. For 77% of the total population diabetes is an incurable chronic disease, for 14% an easily curable disease and 9% DKn/NA. Among those with diabetes, 64% reported total compliance with doctors' recommendations, 5% high, 15% mostly, 8% little, and 5% no compliance. 28% of the population free of diabetes reported a family member with diabetes, 94% of these reported no concealment of the disease in their social behavior.
Conclusion
We believe that the level of awareness of diabetes in northern Greece is satisfactory. Communication to the general population of the main predisposing factors especially obesity and the importance of compliance to treatment in order to optimize treatment and avoid complications are needed

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Insulin resistance among adults with type 1 diabetes mellitus

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Insulin resistance (IR) has been proven to increase the risks for cardiovascular complications in type 2 diabetes mellitus. Recently, IR has also been shown to play a bigger role in the natural history of type 1 diabetes mellitus (T1DM) disease process than is commonly recognized. The objectives of this study are to determine the prevalence of IR among Filipino adults with T1DM and to describe the clinical features of T1DM with IR.
This cross-sectional analytic study recruited 83 adults with established T1DM in Philippine General Hospital. Mixed-meal stimulated C-peptide level was done to confirm the diagnosis of T1DM. IR was determined using estimated glucose disposal rate (eGDR) with the formula of: eGDR = 24.31 (12.22 × waist-to-hip ratio) – (3.29 × 1 if with hypertension or on anti-hypertensive or × 0 if no hypertension) – (0.57 × HbA1c). Subjects with eGDR of ≤7.5 mg/kg per min were considered to have IR.
The prevalence rate of IR was found to be 53%. T1DM subjects with IR were significantly older (29.59 vs 25.59, $P = 0.007$), with longer duration of diabetes (59.7 vs 40.3% with duration of diabetes >5 years, $P = 0.037$), with higher waist-to-hip ratio (0.95 vs 0.93, $P = 0.005$) and with higher rate of hypertension (100 vs 0%, $P = 0.00$) than those without IR.
The study showed a high prevalence rate of IR among Filipino adults with established T1DM. Hypertension, older age, longer duration of disease and a higher waist-to-hip ratio are the features of T1DM with IR.

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Atypical early onset Werner's syndrome

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Introduction
Werner's Syndrome (WS) is a rare autosomal recessive adult onset progeroid disorder characterized by the early onset of aged-appearance and age related metabolic disorders. Patients usually develop normally until the 3rd decades of life.
Case report
We report a 27 years old female who admitted to our clinic at the age of 15 with hyperglycemia. She was diagnosed as diabetes and type 4 dyslipidemia at the age of 7 years. In her family history, her parents were first cousins and she had three healthy brothers.
At her first examination she had bird-like face appearance, short stature and she was overweight. She had global alopecia with gray and thin hair. Her voice was hoarse and hyperkeratosis of skin was present at dermatological examination. She had bilateral cataracts and moderate sensorineural hearing loss. At psychiatric examination she had borderline mental retardation.
She has got severe insulin resistance and hypertriglyceridemia despite therapy with insulin, levothyroxine, gemfibrozil and omega-3. Routine lipid apheresis has been performed to lower the triglyceride levels reaching 5256 mg/dl.
She also had focal segmental glomerulosclerosis, hepatosteatosis, osteoporosis and epilepsia. She had several congenital deformities like rathke's cleft cyst, angiomyolipoma and femoral neck hypoplasia.
Discussion and conclusion
Werner's syndrome may have very early onset and present with severe hypertriglyceridemia and multiple metabolic and congenital abnormalities.

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Potentialities of skin impedance spectroscopy *in vivo* in non-invasive monitoring of glucose level

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Considered are the physiological mechanisms of relationship of the magnitude of blood plasma and interstitial fluid osmolarity conditioned by glycaemia with transmembrane movement of water between intra- and extracellular water compartments of organism. Analysed are the potentialities of impedance spectroscopy in quantitative evaluation of increments of specific volumes of extra- and intracellular fluid of hypoderm tissues occurring due to fluctuations of intercellular fluid glucose level. Substantiated is the possibility of technical implementation of individual systems for non-invasive monitoring of glucose concentration in intercellular fluid on the base of two-frequencies (30 and 1000 kHz) impedancemetry of human skin. Represented are the results of interstitial liquid glucose level calculation performed on the base of transcutaneous measurement of wrist tissues impedance in persons ill with diabetes 1 and 2 and in healthy volunteers. Fulfilled is the comparative analysis of results of glucose level calculation on the base of impedancemetry with the reference data provided by invasive measurements in blood and interstitial liquid by system invasive glucose monitoring Medtronic Guardian Real Time. Revealed is the strong correlation – Pearson's coefficient is higher than 0.8 – between the results of calculated glucose concentration on the base of impedancemetry and reference data obtained by invasive measurements.

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Association of plasma testosterone concentration with cardiovascular risk factors in young Chinese men of type 2 diabetes mellitus

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Introduction
Type 2 diabetes mellitus (T2DM) has the same risk of cardiovascular event with coronary artery disease (CAD). Men with diabetes are characterized by a decrease in circulating testosterone concentrations, and testosterone deficiency have been associated with increased risk of metabolic syndrome and CAD. With the increased popularity of western life style in China, the morbidity of T2DM increased greatly in young Chinese people. This study aimed to investigate the association of plasma testosterone concentration with certain cardiovascular risk factors in young Chinese men of T2DM.
Methods and design
We reviewed 300 aged 18–30 male T2DM patients in the Zhongnan Hospital, Wuhan University through 2010–2011. All the patients were given oral glucose tolerance test (OGTT), insulin release test (IRT), and the detection of serum testosterone, serum lipid, Hs-CRP. All the patients were given electrocardiogram and detection of myocardium enzyme to rule out the acute coronary syndrome.
Results
Serum testosterone was significantly correlated with age ($r=-0.14$), BMI ($r=-0.15$), waist circumference ($r=-0.21$), triglyceride (TG) ($r=-0.23$), Hs-CRP ($r=-0.26$), MBP ($r=-0.12$), and HOMA-IR ($r=-0.24$) (all $P<0.01$). After adjustment for BMI, waist circumference, age and smoking history, the plasma testosterone was still significantly correlated with Hs-CRP, HOMA-IR, and TG. There were 180 patients with smoking history who had lower serum testosterone concentration in compared with non-smokers in this study, but the difference was not significant. There was no significant difference in the plasma testosterone concentration between the patients with or without coronary heart disease family history. Multiple regression analysis indicated that serum testosterone concentration was the independent predictor of HOMA-IR, Hs-CRP, and TG.
Conclusions
Serum testosterone in young T2DM men was significantly and independently correlated with HOMA-IR, Hs-CRP and TG, and might be one of the predictors of cardiovascular disease.

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Leptin and other adipocins in patients with type 2 diabetes

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Aim
To evaluate leptin and other adipocins content in the blood with type 2 diabetes.
Materials and methods
Eighty six patients with type 2 diabetes were examined (59 women and 27 men) middle-aged 58.6 ± 1.04 years old. The control group included 16 patients (eight women and eight men) middle-aged 22.6 ± 0.6 years old. The HOMA-IR, insulin, adiponectin, resistin, leptin, and omentin levels were investigated.
Results
In patients with type 2 diabetes HOMA-IR (6.13 ± 0.8), insulin level (16.91 ± 2.01 mcME/l) is actually higher than in control group (7.78 ± 0.43 and 1.48 ± 0.11 accordingly, $P<0.001$).
Adiponectin level in men with type 2 diabetes was evidently lower (7.5 ± 0.4 μg/ml) than in control group (10.6 ± 0.32 μg/ml, $P>0.05$). Resistin level in type 2 diabetes was statistically higher (10.37 ± 0.55 ng/ml), than in control group (7.46 ± 0.51 ng/ml, $P<0.01$). The lower omentin content in the patient's blood with type 2 diabetes (264.23 ± 12.02 ng/ml) compared with the control group (395.6 ± 5.3 ng/ml, $P<0.001$).
The leptin content in type 2 diabetes men (13.15 ± 1.14 ng/ml) and women (34.88 ± 2.99 ng/ml) is actually higher than in control group (3.59 ± 0.38 ng/ml in men and 9.56 ± 0.67 ng/ml in women, $P<0.001$). The leptin level in type 2 diabetes men positively correlates with insulin level ($r=0.488$, $P<0.05$) and HOMA-IR ($r=0.476$, $P<0.05$), but in type 2 diabetes women with IMB ($r=0.652$, $P<0.001$), WC ($r=0.562$, $P<0.001$) and insulin level ($r=0.334$, $P<0.05$).
Conclusion
The increase of leptin and resistin levels and reduction of adiponectin and omentin levels are noted in type 2 diabetes.

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Leptin, resistin and omentin in patients with the obesity and with impaired glucose tolerance

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Aim
To evaluate the adipocins content in the blood with IGT.
Materials and methods
One hundred and one patients with IGT were examined (86 women and 15 men) middle-aged 62.1 ± 1.2 years old. The control group included 16 patients (eight women and eight men) middle-aged 22.6 ± 0.6 years old. The anthropometric indications (IMB, WC, and WC/TC), HOMA-IR, insulin, CRP, resistin, leptin, and omentin levels were investigated.
Results
All patients with IGT had IMB more than 30 kg/m^2, WC in men was higher than 94 sm, in women – 80 sm, that shows that they have visceral obesity type. HOMA-IR, insulin, CRP levels in patients with IGT is actually higher (4.12 ± 0.28, 16.14 ± 0.93 ng/ml and 4.82 ± 0.63 mg/l accordingly) than in control group (1.48 ± 0.11, 7.78 ± 0.43 ng/ml and 2.67 ± 0.56 mg/l accordingly, $P<0.001$). Resistin level in IGT was statistically higher (9.85 ± 0.41 ng/ml), than in control group (7.46 ± 0.51 ng/ml, $P<0.05$). The lower omentin content in the patient's blood with IGT (271.52 ± 14.92 ng/ml) compared with the control group (395.6 ± 5.3 ng/ml, $P<0.001$). The leptin content in the men's blood (12.2 ± 4.5 ng/ml) and IGT women (40.95 ± 3.04 ng/ml) is actually higher than in control group (3.59 ± 0.38 ng/ml in men and 9.56 ± 0.67 ng/ml in women, $P<0.001$). The leptin level in IGT women positively correlates with IMB ($r=0.612$, $P<0.001$), WC ($r=0.743$, $P<0.001$), insulin level ($r=0.519$, $P<0.001$), HOMA-IR ($r=0.496$, $P<0.001$), CRP level ($r=0.515$, $P<0.01$), and negatively correlates with omentin level ($r=-0.804$, $P<0.001$).
Conclusion
The increase of leptin and resistin levels and reduction of omentin level are noted in IGT in case of having visceral obesity and insulin resistance.

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Quality of care in diabetic patients

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Introduction
Type 2 diabetes mellitus (T2DM) is a chronic disease, with prevalence increasing worldwide and its complications are major causes of early morbidity and mortality. Recent guidelines suggest the individualisation of glycaemic targets and glucose-lowering therapies.
The aim of this study was to determine the quality of care provided to type 2 diabetic patients in our institution, analysing metabolic control, cardiovascular risk factors and prevalence of diabetic complications.
Methods
Transversal study with 423 type 2 diabetic patients, followed at our diabetes clinic.
Results
A total of 423 patients were included in the study, with a mean age of 67 ± 9.4 years. 61.7% were men. Approximately 90% of patients were overweight (41.9% obese). 65.3% had familiar history of T2DM. Mean duration of diabetes was 15 ± 10.5 years and HbA1c levels averaged 7.0 ± 1.2%. 60% had HbA1c ≤7% (40% HbA1c ≤6.5%). Concerning therapeutic regimens: 75% used oral hypoglycaemic agents (OAD) alone (73.4% of these were using two or more agents); 25% were treated with insulin (16% in combination with OAD). 82.3% of the patients had hypertension and 31.2% met the target blood pressure (BP) of 130/80 mmHg. 82.9% had dyslipidaemia and 62.6% met the goal LDL cholesterol level <100 mg/dl. 12% of patients met the combined ADA goal for BP, LDL, and HbA1c. 61.3% of the patients was anti-aggregated.Regarding diabetic complications: 16.7% had retinopathy, 26.4% nephropathy, 23% cardiovascular, and 8.7% cerebrovascular disease.
Conclusions
Metabolic control (HbA1c), BP, and LDL values were favourable in our patients, comparing to other studies. Although, it's challenging to achieve all the goals proposed by international guidelines.

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Lipid profile of patients newly diagnosed with type 2 diabetes in Albania

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Background and aims
Diabetic patients are at increased risk for macrovascular complications and lower extremity amputations. Knowing their metabolic control degree, and lipid profile, through an early intervention, we can reduce their risk factors for cardiovascular disease, and diabetes complications. The aim of our study was to determine the lipid profile of patients newly diagnosed with type 2 diabetes.
Patients and methods
Hundred patients, selected at the outpatient policlinic no. 3 in Tirana, the capital of Albania. All the patients had completed anthropometric measures, HbA1c and lipid profile after a 12-h fast. All the persons younger than 30 years, diabetes diagnosed before 6 months, or uncompleted data were excluded from the study.
Results
We obtained all the data for 75 patients. Males 48 (64%), mean age 55.6 ± 9.32 years, mean BMI 28.7 ± 4.2 kg/m^2, mean HbA1c $7.44\% \pm 2.54$. 47.6% of the patients had a total cholesterol >220 mg/dl, 16% of the patients had triglycerides >250 mg/dl, and 41.3% of them had TG <150 mg/dl, and 64% had the LDL >130 mg/dl. 83.3% of males had HDL <45 mg/dl and 100% of women had HDL <55 mg/dl respectively.
Conclusions
In our study the lipid profile of Albanian patients was somehow different from the common profile of patients newly diagnosed with T2 diabetes. Even in the previous studies we have found a lipid profile with high total cholesterol levels, and especially very low HDL levels, probably due to the sedentary lifestyle, which needs further evaluation, because the metabolic control of our patients was not very bad.

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Glycemic and lipid profile in patients with acute pancreatitis

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Introduction
Early hyperglycemia in acute pancreatitis (AP) is prognostic sign of severe attack. Elevated lipid levels are one of the major risk factors for AP. The objective of this work was to evaluate the glycaemic and lipid profile in patients (pts) with acute pancreatitis, treated in our institution.
Methods
A total of sixty nine pts with AP were admitted to a Department of Internal medicine, from January to December 2012.
At admission, plasma glucose concentration, total cholesterol serum level, triglyceride level, HDL cholesterol and LDL cholesterol level were measured. In patients with no previously known DM, and plasma glucose concentration at admission $\geq$ 7.8 mmol/l (14 pts), the fasting plasma glucose (FPG) and glucose monitoring during hospitalization were performed. In pts with persistent FPG $\geq$6.1 mmol/l, the oral glucose tolerance test was done 1–2 days before hospital discharge.
Results
Of sixty nine patients studied, 39 pts (57%) were female. Average age of pts was 59.9 ± 18.3 years (min 25 years and max 90 years). Total cholesterol serum level was 5.13 ± 3.27 mmol/l (range 2.3–20 mmol/l), triglyceride 3.63 ± 12.03 mmol/l (range 0.4-63.7 mmol/l), HDL cholesterol 1.25 ± 0.48 mmol/l (range 0.5–2.4 mmol/l), LDL cholesterol 2.75 ± 1.25 mmol/l (range 0.7–4.7 mmol/l), plasma glucose concentration at admission 7.74 ± 3.05 mmol/l (range 3.6–20.2 mmol/l), and FPG at discharge 5.65 ± 1.48 mmol/l (range 4.3–10.4 mmol/l). Previously known DM was recorded in 12 pts (17.4%); before admission 6 of them were treated with insulin, five with oral medication, one patient only with diet. According to blood glucose tests, seven pts had newly diagnosed DM type 2 (two pts on diet, three pts oral agents, and two pts requiring insulin at the discharge), and six had new impaired glucose tolerance (IGT).
Conclusion
High percentage of pts with hyperglycemia on admission and newly diagnosed glycemic abnormalities at discharge, suggest the need of early diagnosis and appropriate treatment of this conditions.

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Practical knowledge about diabetes and the risk of death: 9-year observation

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Introduction
Although education is widely regarded as one of the pillars of diabetes treatment, it is relatively little work analysing its effectiveness. Therefore we would like to present our prospective observations.
Method
In 2003–2004, 500 consecutive patients visiting our diabetes education centre put to the test the knowledge of diabetes as 20 questions which focus on practical skills proceedings in diabetes. We did not have a direct affect their further treatment carried out by general practitioners and diabetologists.
Results
Patients who survived had significantly higher number of correct answers (69 ± 22 vs $53 \pm 21\%$, $P<0.001$). Of the 20 questions, there were no differences in only three concerning the definition, diabetic foot prevention, and management of fever. In both groups, more than 80% of those granted the right answers to these questions.
While the same test of knowledge about diabetes, made again after 9 years among 100 patients gave a surprisingly similar result n (68 vs 69%, $P=0.417$).
Interpretation
Knowledge test about diabetes checker practical ability to proceedings in diabetes proved to be an important tool in assessing the risk of death during the next 8–9 years. This does not mean automatically that the possible improvement in diabetes knowledge would translate into longer life of our patients. However, this can be checked in the intervention study, where the directions of education will be determined by the results of the practical test procedure.

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Blood pressure control in type 2 diabetic patients with overweight or obesity in Guangdong Province; a cross-sectional survey

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Objective
To investigate the blood pressure control in type 2 diabetic patients with overweight or obesity in Guangdong Province.
Methods
From August 2011 to March 2012, The 2 diabetic patients with overweight or obesity was recruited from 67 hospitals in 20 cities of Guangdong Province, and received a standard questionnaire. The conditions of Demographic data, clinical examinations, the most recent laboratory assessment, and drug therapy were recorded.
Results
Totally, 4318 questionnaires with complete information were collected. Mean age of patients was 58.81 ± 12.98 years. Mean duration of hypertension was 7.89 ± 7.28 years, and mean BMI was 27.29 ± 2.78 kg/m^2. Among the participants, the prevalence of hypertension was 58.5%, controlled BP was achieved in 23.8% patients dysfunction. Multiple logistic regression analysis showed that older age, obesity, history of hypertension and inadequate glycemic control were the factors related to unsatisfactory blood pressure control rate in investigated patients. Calcium channel blocker (58.0%) and angiotensin-II receptor blockers (43.1%) were the most frequently used medications.
Conclusions
Type 2 diabetic patients with overweight or obesity in Guangdong Province have poor blood pressure control status. Thus, active lifestyle interventions and drug therapy should be taken to reduce the complications and mortality of the population.

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The role of a lifestyle modification in preventing type 2 diabetes mellitus and influence it on changes serum leptin levels

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Aim
The aim of our study is to assess the efficacy of a lifestyle modification including altered diet composition and physical activity in preventing diabetes mellitus type 2 (DM 2) in individuals with impaired glucose tolerance and impaired fasting glucose (IGT/IFG) and influence it on changes serum leptin levels
Materials and methods
The study included 327 patients with IGT/IFG (68 males and 258 females) 25–65 years. Patients were divided into two groups matched by sex, age, weight, and BMI. Research group included 183 patients (32 males and 150 females) who received and carried out individual recommendations of a balanced diet and physical activity. Control group included 144 patients (36 males and 108 females) who did not lifestyle modification. Related to fasting leptin (FL) concentrations by sensitive ELISA.
Results
Patients of the research group demonstrated reduction of body weight ($P<0.01$). They had positive dynamics of FPG and 2-h PG concentrations also ($P<0.001$). Persons of the control group had significant increase in weight and BMI and FPG and 2-h PG concentrations elevated ($P<0.05$). The main novel finding was that median serum leptin in research group decreased on -23.9% ($P<0.01$) and increased in control group on $+27.6\%$ ($P<0.01$) among subjects with IGT Among patients of the research group was a reduction of new care DM 2 by 11.9% and an increase in the control group by 35.1%.
Conclusion
Thereby, lifestyle modifications lead to reduction not only fasting plasma glucose, postprandial glucose concentrations but and fasting leptin concentrations in individuals with IGT/IFG.

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Signs of accelerated carotid atherosclerosis assessed by MRI in newly diagnosed type 2 diabetes

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Introduction
Type 2 diabetes is associated with accelerated atherosclerosis, which causes macro-vascular complications such as cerebral infarctions, myocardial infarctions, and peripheral vascular disease. We aimed to investigate whether there are differences in the morphology of atherosclerosis in the carotid arteries assessed by MRI in newly diagnosed type 2 diabetic patients compared to non-diabetic control subjects.
Methods
Hundred type 2 diabetic patients diagnosed within the last 5 years and 100 age- and gender-matched non-diabetic control subjects underwent magnetic resonance imaging of carotid arteries bilaterally in a 1.5 Tesla Phillips Intera MRI scanner with a dedicated carotid coil. Scans were performed with four different contrast weightings and analysed in a software tool to assess atherosclerosis morphology.
Results
One hundred and fifty four carotid arteries in the diabetes group and 178 carotid arteries in the control group were available for analysis. In diabetic patients the minimal lumen area was 17.6% smaller ($P<0.001$) and maximal normalized wall index was 3.0% higher ($P=0.038$) than in the control subjects. This remained significant after adjustment for LDL-cholesterol and smoking habits (minimal lumen area, $P<0.001$ and maximal normalized wall index, $P=0.012$). Sub-analysis of those measures revealed pronounced differences between diabetic patients and control subjects among the youngest 50% of the participants.
Conclusion
Clear signs of accelerated carotid atherosclerosis assessed by MRI were found at a very early stage of type 2 diabetes, despite good regulation of hyperglycemia, hyperlipidemia and blood pressure. There is an effect on the arterial wall remaining after correction of classical cardiovascular risk factors, suggesting a legacy effect.

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Cardiovascular risk management in the patients with type 2 diabetes mellitus

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Current guidelines on cardiovascular (CV) disease prevention recommend targeted management after assessment of CV risks using many of available method even if the patient is asymptomatic. This study was performed to explore how CV high-risk is differently detected between two distinct methods: non-invasive test (NIT) and risk calculation and how the awareness of CV high-risk impacts physician and patient behavior for risk management in diabetes patient. A prospective, observational study was carried out in 22 hospitals in Korea. 622 type 2 diabetes patients aged ≥ 40 years were assessed by carotid ultrasound (CUS) and United Kingdom Prospective Diabetes Study (UKPDS) risk engine. CV high-risk from CUS was defined as carotid intima-media thickness ≥ 1 mm or plaque presence. Before and 6 months after the test, patients completed a questionnaire on health-related behaviors and physicians collected data on treatment patterns via chart review.
Among 622 (mean age, 60.02 ± 9.50 years), 271 (43.5%) and 66 (10.6%) patients were stratified as CV high-risk from CUS and UKPDS respectively. Approximately 40% of patients at moderate and low risk from UKPDS were determined as high-risk from CUS. The awareness of high-risk from CUS altered physician's treatment patterns ($P=0.021$) for managing major CV risk factors: blood pressure (BP) and lipid. Along with CUS, risk levels by UKPDS also impacted physician's behavior: more changes of treatment pattern in high than in low risk level for BP (12.0 vs 15.6%) and lipid (13.8 vs 21.6%). Patients noted increased health-related behaviors: smoking cessation and dietary changes ($P<0.005$ respectively) in 6-month follow-up than before CUS and bigger in high-risk.
This study identified NIT could detect more CV high-risk patients than risk calculation. However, awareness of CV high-risk itself has a positive impact on physician and patient behavior, regardless of assessment methods. Therefore, assessing CV risks using varied methods with the patient could be better for risk management in diabetes mellitus.

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P404

Circulating endothelial progenitor cells as a predictor of clinical outcomes in diabetic patients with symptomatic chronic heart failure

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Background
Recent evidence has defined that circulating endothelial progenitor cells (EPCs) might have a pivotal role in the presence of atherosclerosis, chronically diseased vessels or following acute vascular injury. We hypothesized that diabetes mellitus (DM) may be related to worsening of ischemic chronic heart failure thereby suppression of mobilization of bone marrow-derived EPCs.
The aim of this study was to evaluate predict value of circulating EPCs in ischemic chronic heart failure patients with 2 type DM in long-term follow-up.
Methods
sixty eight moderate-to-severe ischemic chronic heart failure (CHF) subjects (36 with 2 type DM, left ventricular ejection fraction = 42.68% (95% CI 36–51%) aged 46–62 years were enrolled to the study. Vessel-wall and plaque geometrical and compositional parameters were measured on contrast-enhanced CT angiography. Immunostaining and flow cytometric technique (FCT) were used for predicable distinguish cells subsets depended on expression of CD14, CD34, Tie-2, CD45, and VEGFR2. Circulating EPCs are defined accordingly ISHAGE criteria as CD34/VEGFR2 positive cells in absent CD45 expression. 100 000 events were analyzed from each tube at baseline of the study. Mononuclear cells

were cultured for functional analysis (CFUs) after FCT. Standardized cell counts were presented as a percentage of total leukocytes, which were identified as the total number of all CD45+ cells. Observation period for the patients was 6 months.
Results
Analysis of obtained outcomes have been shown a significantly decreasing of the total CFU count and also circulating CD34+ subsets level: CD34+ CD45− VEGFR2+, and CD34+ CD45− Tei-2+ VEGFR2+ cells in CHF patients with 2 type DM when compared with subjects without one ($P<0.001$). Second type DM (HR=6.20, 95% CI 3.11–14.10, $P=0.009$), and lower CD34+ CD45− VEGFR2+ (HR=4.64, 95% CI 1.99–7.36, $P=0.004$) and lower CD34+ CD45− Tei-2+ VEGFR2+ (HR=0.58, 95% CI 3.15–12.10, $P=0.007$) were independent prognostic variables for cardiovascular outcomes (composite point included hospitalization rate, mortality rate, all cardiovascular events, worsening of CHF) within 6 months. Results did not change after adjustment for age, sex, BMI, smoke, hypercholesterolemia, arterial hypertension, NYHA functional class of CHF and previous myocardial infarction.
Conclusions
A lower of circulating EPCs defined as CD34+ CD45− VEGFR2+, and CD34+ CD45− Tei-2+ VEGFR2+ subsets cells in ischemic CHF patients with 2 type DM might have a high predict value for further cardiovascular outcomes. These findings can be taken into consideration as supporting of hypothesis about that such cellular biomarkers can reflect potential vascular repair insufficiency in diabetic patients with CHF.

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P405

Correlations between umbilical and maternal adiponectin as well as neonatal birthweight

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Introduction
Adiponectin (AdipoQ) is a known insulinsensitizing adipokine which is also present in fetal circulation. It has been postulated to play a role in the regulation of fetal growth and development together with other adipokines. The aim of the study was to determine fetal adiponectin and find its correlations with the neonatal birthweight and maternal AdipoQ.
Patients and methods
The study included 36 women with singleton pregnancy (20 diagnosed with GDM) and their 36 term neonates born by elective cesarean section. Women with chronic disorders were excluded from the study. Laboratory assessment included the routine tests (glucose and insulin concentration) and AdiopoQ level in the maternal blood at delivery and in the umbilical blood of the neonates. AdipoQ levels were determined by human adiponectin ELISA, high sensitivity, and BioVendor.
Results
Maternal AdipoQ was lower in the study group than in the control group, the median with interquartile range were 4.9 ng/ml (4.4–5.8), 5.9 ng/ml (5.1–8.0) respectively ($P<0.005$); umbilical AdipoQ was significantly higher than the maternal one in both groups 21.9 ng/ml (19.4–29.4) and 31.1 ng/ml (28.1–41.4). Newborns of GDM mothers had significantly lower umbilical AdipoQ levels than those of healthy mothers ($P=0.007$). No gender differences were found between the neonates. AdipoQ in women with GDM significantly negatively correlated with glycemia at 120 min of OGTT in pregnancy, with insulin, HOMA-IR, HOMA-B, whereas it positively correlated with the QUICKI values. Umbilical AdipoQ significantly negatively correlated with the maternal glycemia at 120 min of OGTT only in women with GDM. No correlations between the fetal AdipoQ and the maternal one or the neonatal birthweight were found.
Conclusions
Umbilical AdipoQ is significantly higher than the maternal one, but it is maternal adiponectin-dependent and shows no significant correlation with the neonatal birthweight. Maternal hyperglycemia may be a vital factor modulating fetal AdipoQ concentration.

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P406

The relationship between glycemic control and BNP levels in diabetic patients

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Background
Glycemic control affects cardiovascular risk factors positively. The purpose of this study was to assess BNP levels in patients with poor control diabetes before specific treatment for hyperthyroidism and after glycemic regulation was achieved. The study was performed in a prospective design.
Methods
The study population consisted of 79 consecutive diabetic patients with poor glycemic control. All subjects underwent transthoracic echocardiography. Levels of fasting plasma glucose, HbA1c, lipid parameters, BNP were measured before the onset of the treatment and after glycemic regulation was achieved.
Results
A significant decrease in BNP (95.0 (4.0–1807) ng/l vs 52.0 (2.1–987.0) ng/l, $P<0.001$) levels were observed, after improving glycemic control. The decrease in BNP levels was positively correlated with the decrease in HbA1c ($r=0.345$; $P=0.003$) and fasting plasma glucose ($r=0.366$; $P=0.002$). There was no correlation between the decrease in BNP levels and the lipid parameters ($P=$NS).
Conclusion
We conclude that poor glycemic control may cause high BNP measurements which can lead to misdiagnosis of congestive heart failure. We suggest that glycosylated hemoglobin and fasting plasma glucose should be checked in patients with high levels of BNP in diabetes.

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P407

Diagnostic and prognostic value of system matrix mettalloproteinases-1 in patients with diabetes mellitus 2 type

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Non-alcoholic fatty liver disease (NAFLD) develops in 75–90% of patients with diabetes mellitus 2 type (DM2) and obesity (Ob). Collagen degradation is regulated by the activity of interstitial collagenases, especially matrix metalloproteinases 1 type (MMP-1). It is necessary to determine levels of tissue inhibitor of metalloproteinases-1 (TIMP-1), which regulates activity of MMP-1.
Aim
Optimisation of diagnosis and specification of prognosis of NAFLD in patients with DM2 by determination of distant fibrosis markers: MMP-1 and TIMP-1.
Methods
Four groups (grp) of patients (pts) were investigated: first grp included 30 DM2 pts without NAFLD, second grp had 20 NAFLD pts without DM2, third grp had 30 DM2 pts with NAFLD without Ob, fourth grp had 30 DM2 pts with NAFLD and Ob and also 20 healthy persons were examined. The diagnosis of NAFLD was confirmed by histology and ultrasound. Plasma levels of proMMP-1, TIMP-1 by ELISA method were studied.
Results
Progressive and significant increase in levels of proMMP-1, TIMP-1 both in comparison with control grp, and between grps, were revealed indicating increased severity of DM2 when accompanied by NAFLD and Ob.

Table 1

		Groups of patients ($n=120$)			
Plasma levels	Control group ($n=20$)	1 group ($n=30$)	2 group ($n=20$)	3 group ($n=30$)	4 group ($n=40$)
proMMP-1 (ng/ml)	1.4±0.05	2.0±0.096*	2.3±0.1*	3.1±0.12*,†,‡	3.6±0.12*,§
TIMP-1 (ng/ml)	373.0±1.6	396.0±2.8*	407.0±1.7*,†	420.0±2.5*,†,‡	442.0±2.4*,§

*$P<0.05$ vs control grp, †$P<0.05$ vs 1grp, ‡$P<0.05$ vs 2 grp, and §$P<0.05$ vs 3 grp.

Conclusions
Increase of levels of proMMP-1, TIMP-1 in DM2 pts were caused by damage of liver and contributed to clinical outcomes. Evaluation of proMMP-1, TIMP-1 in

pts significantly improve diagnostic of NAFLD. High MMP-1 levels were associated with the risk for development of NAFLD and useful as a noninvasive marker of NAFLD.

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P408

Fibroblast growth factor 21: a new predictor of cardiovascular events in type 2 diabetes

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Introduction
The course of type 2 diabetes (DM2) is linked with increased fibroblast growth factor 21 (FGF21) concentrations, however little information is available on the prognostic significance of FGF21 in DM2. The aim of the study was to assess whether FGF21 may be a predictor of cardiovascular (CV) events in patients with DM2.
Patients and methods
The study comprised 87 patients with DM2, aged 57–66 years, with the median duration of diabetes of 10 years, who were referred to the Department of Endocrinology for routine annual metabolic assessment. All the patients underwent clinical and laboratory assessment at the time of enrolment; next, the study group was observed prospectively during the period of 24 months. Serum FGF21 levels were assessed with 'Human FGF21 ELISA' BioVendor. During a follow-up, overall mortality, CV mortality, and CV nonfatal events were registered. Cox proportional hazards regression assessed adjusted differences in CV morbidity and mortality risk.
Results
Patients were stratified according to serum FGF21 levels less than or equal to and greater than the median value of 240.7 pg/ml. The groups showed no significant differences at baseline in gender distribution, diabetes duration, insulin therapy, BMI, biochemical profiles and previous CV events. At 24-month follow-up, 21 (24.1%) patients experienced a nonfatal CV event. A significantly ($P=0.0013$) higher incidence of the combined end point of CV morbidity and mortality was observed in the FGF21 >240.7 pg/ml group. In the multivariate Cox proportional hazards regression model, the presence of FGF21 greater than the median value was associated with a significant increase in the risk of the combined end point of CV morbidity and mortality (HR: 4.7, 95% CI 1.67–13.24).
Conclusion
The increased FGF21 concentrations may serve as a predictor of CV events and provide a useful tool for stratification of prognosis in DM2 patients.

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P409

Diabetes and hyperglycemia: relation with clinical outcome in the community acquired pneumonia

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Background
Community acquired pneumonia (CAP) is a common condition and a major cause of morbidity. Diabetes mellitus (DM) increases the risk and complications of infectious diseases. However, it is necessary to clarify if DM and glycemia at the time of presentation are prognostic factors in patients with CAP.
Objectives
To evaluate the relationship between DM/glycemia at the time of presentation and complications, length of stay and mortality in patients with CAP, and the relationship between glycemic control and complications in diabetics.
Methods
Observational, analytical, and retrospective study of adults admitted to our Hospital between October/2011 and March/2012, with CAP. Electronic clinical data were analyzed and telephone calls were done to assess mortality at 30 and 90 days. The χ^2, Mann–Whitney, Kruskal–Wallis tests and logistic regression were used.
Results
Of the 440 included patients, 51.1% were women, 83.1% elderly and 29.3% had a prior diagnosis of DM. Of these, 48.8% had HbA1c measured (median 6.8%, 25th percentile: 6.3%, and 75th percentile: 7.8%). The median glucose was 134 mg/dl (P25: 111 mg/dl and P75: 176 mg/dl). It was shown that patients with DM were older ($P=0.002$), had higher severity of pneumonia, assessed by CRB-65 ($P=0.025$), more complications ($P=0.001$), and longer length of stay ($P=0.001$). DM proved to be a predictor of complications ($P=0.008$). There was no association between DM and mortality, nor between HbA1c levels and complications, length of stay and mortality. Moreover, it was verified a gradual increase of days of stay for higher glucose levels at admission ($P=0.016$) and a trend towards complications in patients with hyperglycemia. However, there were no statistically significant differences between glucose levels and mortality.
Conclusion
DM and hyperglycemia on admission are associated with adverse outcome in CAP. Both are associated with prolongation of stay and DM predicts complications from CAP.

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P410

iNOS, eNOS, and XOR involvement in hyperglycaemia-induced kidney injury in rats with streptozotocin diabetes mellitus

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Introduction
Diabetic nephropathy is a major complication of diabetes mellitus. Involvement of abnormal nitric oxide (NO) production, both via nitric oxide synthase (NOS) dependent and independent pathways (for example, xantine oxoridoreductase (XOR)) has been discussed. New compounds to treat diabetic nephropathy are searched intensively, some novel dihydropirine class drugs (DHP) have been proposed for this purpose.
Aim
To study the changes in NO concentration and expression of NOS isoforms and XOR in kidneys of diabetic rats in the early phase of diabetic nephropathy and under effect of DHP etaftorone.
Methods
Diabetes mellitus in rats was induced by streptozotocin (STZ) 50 mg/kg, i.v., after a week rats were treated by etaftorone 0.5 mg/kg *per os* for three consecutive days. Production of NO in kidneys was monitored by means of ESP spectroscopy. iNOS, eNOS, and XOR mRNA and protein expression in kidneys were detected by qRT-PCR and immunohistochemistry correspondingly.
Results
Development of STZ DM was followed by a significant increase of NO production in the kidneys, which could be attenuated by etaftoron (control: 2.64 ± 0.97 ng/g tissue, STZ: 15.04 ± 2.04 ng/g tissue, and STZ+etaftoron: 5.52 ± 1.089 ng/g tissue). In STZ group, XOR expression was increased (STZ 27 ± 7 vs control 8 ± 2 cells/mm^2; $P=0.002$.), and normalized by etaftoron (STZ+etaftoron: 9 ± 3 cells/mm^2 vs STZ: 27 ± 7 cells/mm^2; $P=0.0006$). Similar, kidney iNOS protein expression increased in STZ group and was normalized by etaftoron (STZ: 29 ± 15 cells/mm^2 vs control 11 ± 4 cells/mm^2; $P=0.004$; STZ+etaftoron 13 ± 6 cells/mm^2, $P=0.04$ vs STZ). eNOS-expression decreased in diabetic rat kidneys, etaftoron attenuated the decrease.
Conclusions
Diabetic state provokes increase of NO production in rat kidneys shortly after diabetes induction and seems to be associated with hyperexpression of iNOS and XOR. These changes can be attenuated by DHP class drug etaftoron.

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P411

Advanced glycation end-products inhibit insulin signaling in human granulosa cells

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Introduction
AGEs have been shown to be accumulated in granulosa cell layer from human ovaries in normal and in women with polycystic ovarian syndrome (PCOS) and insulin resistance. AGEs interfere with insulin signaling pathways of several target tissues and are implicated in insulin resistance mechanisms. There is

evidence that within the ovary these pathways are also important for glucose metabolism and normal folliculogenesis. The effect of human glycated albumin (HGA) either alone or in combination with insulin was investigated in human ovarian granulosa cells (KGN) relative to Akt activation and glucose transporter 4 (Glut-4) translocation.
Method
KGN cells were cultured with insulin (100 ng/ml) or HGA (0.2 mg/ml) and insulin combined with HGA for 1 h. Activation of Akt was assessed in all conditions followed by the analysis of Glut-4 translocation from the cytoplasm to the membrane compartments of KGN cells. LY294002, a specific PI3K inhibitor was applied to define PI3K-mediated phosphorylation of Akt.
Results
Insulin treatment induced a six-fold increase in p-Akt levels in human granulosa cells compared to basal levels ($P<0.001$) but the combined treatment of insulin and HGA inhibited the insulin-mediated Akt phosphorylation. PI3K inhibitor was able to attenuate Akt phosphorylation indicating that insulin-stimulated Akt activation was PI3K-dependent.
In addition, insulin increased glycosylated Glut-4 variants in both cytosolic and membrane compartments compared to basal levels ($P<0.001$). Moreover, HGA increased Glut-4 in the cytosolic fraction while it severely reduced Glut-4 presence in membrane fraction ($P<0.001$). Combined treatment of insulin and HGA increased cytosolic fraction even further and remarkably reduced Glut-4 translocation to the membrane ($P<0.001$).
Conclusions
AGEs presence in the ovary associated to reduced glucose uptake by granulosa cells potentially affects follicular growth and contributes to the ovarian dysfunction observed in insulin-resistance states such as PCOS.

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P412

Diabetic polyneuropathy course clinical features of patients with various age of diabetes demonstration

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Aim
To single out polineuropathy development risk group.
Materials and methods
Four hundred and fifty patients with a more than 5 years diabetes experience were examined.
For verification of a diabetic polyneuropathy the electroneuromyography was made. By a method of the immunofermental analysis neurospecific S100 protein level was defined. Data are presented in Me (Q25; Q75) format. The importance of distinctions was estimated by Mann-Whitney's criterion.
Results
At 77.3% of patients lesion n. Suralis on axonal type is confirmed. The correlation analysis revealed a direct significant correlation of level of S100 with patient's age (R Spearman $=0.30$; $P=0.02$) and diabetes duration (R Spearman $=0.22$; $P=0.04$). At 25.0% of patients axonal antibodies level increase is revealed. Patients with high level of S100 had the disease for more than 10 years. To single out polineuropathy development risk group the examined patients were divided into groups depending on age of demonstration. Patients with diabetes demonstrations age below 14 years (1st group) and from 20 to 30 years (the 2nd group) had the smallest indicators of S100. Levels of neurospecific protein are 24, 00 (21.06; 30.37) ng/l and 31.84 (24.98; 38.20) ng/l respectively and didn't differ significantly. Patients with more then 40 years diabetes manifestation have 51.11 (36.94; 61.03) ng/l level of S100 which made significant statistical difference with level of (U 360.50; $R<0.005$) of 1st and 2nd group patients.
Conclusions
Level of axonal antibodies is an early laboratory prognostic marker of development of a sensory diabetic polyneuropathy at patients with diabetes.

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Cardiovascular risk and prevalence of metabolic syndrome in LADA patients depending on thyroid autoimmunity presence

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Background
Latent autoimmune diabetes in adults (LADA) is a form of autoimmune diabetes with a slower decline of β cell function and distinct features from type 1 and type 2 diabetes. LADA is also a very heterogenic group. Frequently LADA is associated with other autoimmune diseases especially endocrine diseases.
Aim
To evaluate prevalence of thyroid autoimmunity (TA) and its impact on cardiovascular risk and metabolic syndrome in LADA patients.
Materials and methods
We evaluated 104 (57 females/47 males) patients with LADA (age at onset of diabetes above 30 years, presence of pancreatic antibodies, no need of insulin at least 6 months after diagnosis). Clinical and laboratory data were obtained: weight, BMI, blood pressure, waist, total cholesterol, triglycerides, HDL-cholesterol, and thyroid peroxidase antibodies (TPOAb). Presence of metabolic syndrome (MetS) was evaluated according NCEP/ATP III criteria. Patients were divided in group A with TA and group B without TA.
Results
Mean age of patients was 50.53 ± 11.41 years, mean age at onset of diabetes was 44.03 ± 10.13 years, and mean duration of diabetes was 6.5 ± 4.15 years. TA evaluated by TPOAb was present in 30 patients (28.8%). Triglycerides were 115.17 ± 87.8 mg/dl in group A vs 189.7 ± 115 mg/dl in group B ($P=0.002$), HDL cholesterol was 54.4 ± 11.9 mg/dl in group A vs 45.08 ± 14.4 mg/dl in group B ($P=0.002$), total cholesterol was 186.23 ± 40.4 mg/dl in group A vs 210.18 ± 59.4 mg/dl in group B ($P=0.04$), systolic blood pressure was 127.5 ± 17.9 mmHg in group A vs 136.22 ± 26.2 mmHg in group B ($P=0.05$), dyastolic blood pressure was 72.5 ± 14 mmHg in group A vs 78.3 ± 14.1 mmHg in group B ($P=0.05$), prevalence of MetS was 46.6% in group A vs 64.8% in group B ($P=0.01$); no statistical difference was observed for BMI.
Conclusion
TA identifies a particular phenotype with lower cardiovascular risk and prevalence of metabolic syndrome among LADA patients.

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Endothelial dysfunction in type 2 diabetic patients; its relation with level of albuminuria and frequency of the microvascular complications

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Introduction
Both endothelial dysfunction and albuminuria have been shown to be related to cardiovascular risk. We aimed to investigate relations between endothelial dysfunction, albuminuria and frequency of the microvascular complication in type 2 diabetic patients.
Method
Forty type 2 diabetic patients (24 microalbuminuria and 16 macroalbuminuria) with a mean age of 57.2 ± 10.8 years and 25 healthy control subjects with a mean age of 54.1 ± 9.6 years were included in the study. Endothelial function was assessed by brachial artery flow-mediated dilatation (FMD) in all subjects using high-resolution ultrasonography. The patients were evaluated in terms of microvascular complications, clinical and demographic features. Fasting plasma glucose and A1c levels were measured. Urinary albumin was assessed in 24-h urine sample.
Results
The mean duration of the diabetes was 89.9 ± 90.9 months and the mean A1c level was $10.12\pm1.2\%$ in the diabetic group. All patient had either microalbuminuria (60%) or macroalbuminuria (40%). Retinopathy was observed in 27.5% and neuropathy in 57.5% of patients. Lower FMD and nitroglycerine-mediated dilatation were observed in diabetic patient compared to those of controls (4.6 ± 2.0 vs $10.5\pm3.9\%$, $P<0.0001$; 15.3 ± 5.8 vs $18.3\pm4.6\%$, $P=0.016$ respectively). The correlation analysis of the diabetic patients revealed that FMD was negatively correlated with the frequency of the

complications($r=-0.33$, $P=0.037$). Patients with macroalbuminuria had lower FMD compared to the patients with microalbuminuria (2.9 ± 0.7 vs $5.6\pm1.9\%$, $P<0.0001$). Similarly, patients with retinopathy had lower FMD compared to the patients without retinopathy (3.3 ± 1.3 vs $5.0\pm2.0\%$, $P=0.006$). FMD was negatively correlated with the level of albuminuria ($r=-0.43$, $P=0.006$).
Conclusion
Endothelial dysfunction is seems to be linked to the level of albuminuria, and correlated with the frequency of the complications in type 2 diabetic patients

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P415

Association between type 1 diabetes and oral health status

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Aims
We assessed periodontal status in patients with type 1 diabetes and healthy individuals in relation to their glycemic control, smoking, and inflammatory biomarkers.
Methods
Periodontal status was examined in 107 patients with type 1 diabetes and 40 controls, using oral hygiene index (OHI), community periodontal index (CPI) and teeth number. CPI values of 0–2 and 3–4 were classified as non-periodontitis and periodontitis respectively. Blood samples were analyzed for fasting glucose, HbA1c, C-reactive protein, fibrinogen, interleukin 1, and tumor necrosis factor-α (TNF-α).
Results
Periodontitis was found in 15.0% of the controls and 57.9% of diabetic patients, including 40.0% of these with good metabolic control (GMC, HbA1c $\leq$6.5%) and 59.5% of those with poor metabolic control (PMC). Severe periodontitis was much frequent in the PMC than in the GMC group (26.0 vs 20.0%), and than in the controls (5.0%). The PMC patients had lower number of sextants with CPI 0 ($P<0.001$) and higher number of sextants with CPI 3 ($P<0.001$) and CPI 4 ($P<0.01$), as well as lower teeth number ($P<0.05$) in comparison with the controls. The patients with peridontitis had higher fibrinogen ($P<0.01$) and TNF-α ($P<0.001$) concentration, as well as higher OHI ($P<0.001$) than had the patients without peridontitis. The number of sextants with CPI 0 correlated negatively with fibrinogen and TNF-α levels, whereas the number of sextants with CPI 3 correlated positively with TNF-α and fasting glucose level.
Conclusions
Our results suggest that poor metabolic control of diabetes together with smoking and inadequate oral hygiene increase the risk of severe periodontal destruction in patients with type 1 diabetes.

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P416

Lower incidence of severe hypoglycaemia during pregnancy in a recent cohort of women with type 1 diabetes followed in a routine care setting

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Introduction
To investigate whether the incidence of severe hypoglycaemia in pregnant women with type 1 diabetes can be reduced without deteriorating pregnancy outcomes in a routine care setting.
Methods/design
A new cohort (2009–2011) of 59 women receiving intensified education about the risk of severe hypoglycaemia in pregnancy was compared with an old cohort (2004–2006) of 108 women. The women entered the study at median 8 (range 5–13) weeks. Severe hypoglycaemia (requiring assistance from others) was prospectively reported in structured interviews.
Results
In the new vs old cohort HbA1c was comparable at inclusion (6.8% (5.6–10.7) vs 6.6% (4.9–10.5), $P=0.25$). During pregnancy severe hypoglycaemia occurred in 25% ($n=15$) vs 45% ($n=49$), $P=0.01$, corresponding to an incidence of 1.3 vs 2.5 events/patient-year, $P=0.04$. Repeated severe hypoglycaemia occurred in 7% ($n=4$) vs 31% ($n=34$), $P=0.0003$. At inclusion a higher proportion of women in the new vs old cohort were on insulin analogues (rapid-acting 100 vs 44%, $P<0.0001$; long-acting 53 vs 6%, $P<0.0001$) and insulin pumps (24 vs 5%, $P=0.0002$). Insulin dose at 8 weeks was lower in women on multiple daily injections in the new vs old cohort (0.67 IU/kg (0.3–1.4) vs 0.77 (0.4–1.7), $P=0.02$) and similar in women on insulin pumps (0.54 IU/kg (0.4–1.1) vs 0.59 (0.3–0.9), $P=0.85$). Pregnancy outcomes were similar in the two cohorts. At multivariate logistic regression analysis, insulin dose at 8 weeks (OR 8.2 (95% CI: 1.6–41.9), $P=0.01$), severe hypoglycaemia in the year preceding pregnancy (6.0 (2.6–13.7), $P<0.0001$) and impaired hypoglycaemia awareness (4.8 (2.2–10.3), $P<0.0001$) were associated with severe hypoglycaemia.
Conclusion
A lower incidence of severe hypoglycaemia in pregnancy without deteriorated pregnancy outcomes was observed in a routine care setting. Lower insulin dose in early pregnancy and focus on high-risk patients may contribute.

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Association between periodontal disease and vitamin D status in a type 1 diabetic population

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Background and aims
Periodontal disease (PD) is a chronic inflammatory condition where the key feature is the alveolar bone loss. Diabetes mellitus is a major risk factor in the occurrence of PD and vitamin D has been found to play a role in the development of diseases such as PD for its immunomoduratory and anti-inflammatory effects. The purpose of this study was to evaluate the relationship between PD and vitamin D levels in type 1 diabetic patients.
Subjects and methods
We conducted a prospective longitudinal survey since December 2010–December 2011. Type 1 diabetic patients aged between 18 and 65 years were recruited. Their periodontal status were examined, metabolic parameters were registered and 25OH(D), 1,25OH(D), and PTH were measured.
Results
A total of 59 consecutive type 1 diabetic patients were included. Periodontal examination showed: no PD in 24%of patients, gingivitis in 3%, initial PD in 25%, moderate PD in 17%, severe PD in 24% and previously treated PD in 7%. PD was significanty associated with age and systolic pressure. Serum 25(OH)D concentrations were low ($<$20 ng/ml) in 34.7% of patients, 2% of patients had levels below 10 ng/ml. Levels of 25(OH)D were inversely associated with PTH ($r=-0.521$; $P<0.01$) and directly associated with 1,25(OH)D ($r=0.369$, $P<0.05$). In regression analysis the only variable that showed to be significantly associated with the degree of PD was 1,25(OH)D, being high levels protectors of PD (OR: 0.953, 95% CI: 0.910–0.998). This means that a decrease of 10 pg/ml increases the risk of PD at 2.69 compared to individuals with 1,25(OH)D normal levels.
Conclusions
i) We found in this study high prevalence of PD in type 1 diabetic patients, only 24% of patients do not have any degree of PD.
ii) Low serum 1,25(OH)D concentrations may be associated with increased PD severity.
iii) Future studies are needed to prospectively assess the beneficial effect of vitamin D of periodontal disease.

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P418

The role of cardio metabolic markers to predict carotid intima media thickness in type 2 diabetic patients

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Introduction
The aim of this study was to investigate the cardiovascular risk factors which could predict vascular thickness in diabetic patients who did not have clinical symptom of atherosclerotic vascular disease
Method
A total of 101 type 2 diabetic patients (48 males and 53 females) with a mean age of (53.6 $\pm$ 8.4 years) were recruited in this study. CIMT were measured by B-mode ultrasonography and ankle brachial pressure index (ABI) was measured with a Doppler ultra-sound. Linear regression analysis was performed with CIMT as dependent variable and ABI and cardio metabolic risk factors as independent variables.
Result
We found that, CIMT were associated with blood pressure ($\beta = -0.37$, $P = 0.01$), waist circumference ($\beta = -0.21$, $P = 0.03$), TG ($\beta = -0.12$, $P = 0.05$), and ABI ($\beta = -0.1$, $P = 0.07$). Subsequent adjusted regression model showed, blood pressure ($\beta = 0.37$, $P \leq 0.05$), waist circumference ($\beta = -0.21$, $P < 0.05$), and TG ($\beta = 0.24$, $P < 0.007$) were identified as independent predictorsof CIMT in diabetic patients and the effect of ABI didn't exist anymore.
Conclusion
In conclusion, the present study showed that insulin resistance index and HbA1C were not potential predictors of cardio vascular thickness in type 2 diabetic patients who exposed to diabetes at least for 9 years. While waist circumference, systolic blood pressure and triglyceride were associated with CIMT in these patients.
Key Words
Carotid intima media thickness (CIMT), ankle brachial pressure index (ABI), cardio metabolic risk factors, Insulin resistance, HbA1c, type 2 diabetes.

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P419

Improvement in sympathetic cardiac autonomic functions after 6 months of comprehensive yogic breathing program in patients with diabetes

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Aims and objectives
To assess the effect of Comprehensive Yogic Breathing Program on glycemic control, quality of life, and cardiac autonomic functions in diabetes.
Material and methods
This is a prospective randomized controlled intervention trial. 120 diabetes patients who were having HbA1c between 6 and 8% for atleast 6 months were selected. Oral drug dose was adjusted to keep the HbA1c between 6 and 8. Plasma fasting glucose, post prandial plasma glucose, HbA1c, quality of life, and cardiac autonomic functions were assessed in 120 diabetics. Patients were randomized into two groups, one group receiving standard therapy for diabetes ($n = 56$) and the other group receiving standard therapy for diabetes and comprehensive yogic breathing program ($n = 64$). Standard therapy included advice on diet, walk, and oral antidiabetic drugs. Comprehensive yogic breathing program is an interactive session in which Sudarshan kriya yoga followed by pranayam is taught by certified teacher. Change in fasting, post prandial plasma glucose, HbA1c, and quality of life were assessed. Cardiac autonomic function tests were done before and 6 months after intervention.
Results
There was a significant improvement in psychological ($P = 0.006$) and social domains ($P = 0.02$) and total quality of life ($P = 0.02$) in the group practicing comprehensive yogic breathing program as compared to the group following standard therapy alone. The improvement in sympathetic autonomic function was statistically significant ($P = 0.009$) in the group following breathing program, while the change in standard group was not significant ($P = 0.06$). The fasting plasma glucose and HbA1c comparable in both groups. The post prandial plasma glucose, decreased significantly in the group practicing breathing programme.
Conclusion
There was signficant improvement in postprandial plasma glucose, sympathetic cardiac autonomic functions and quality of life in the group which received comprehensive yogic breathing program.

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P420

Relationship between metformin treatment and hyperlactacidemia diagnosed at the emergency room

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Introduction
Renal function and glucose lowering therapy, particularly metformin, influence serum lactate concentration in patients with type 2 diabetes (T2D). However, seems to be an acute precipitating event that triggers hyperlactacidemia and metabolic acidosis. This study aims to assess prevalence of hyperlactacidemia in T2D patients who presented at emergency room (ER), calculate relative risk of hyperlactacidemia in diabetics under metformin, identify predictive factors for high lactate concentration and determine influence of hyperlactacidemia in patients' prognosis.
Methods
Prospective study of consecutive T2D patients that attended our hospital's ER. Exclusion criteria: organ transplantation, pregnancy, metastatic malignancy, HIV, pheochromocytoma, alcoholism, convulsions, severe hypoxemia, hemodynamic instability, severe hepatic dysfunction or any known cause of metabolic acidosis. Selected as controls non-diabetic patients observed during same period. Studied variables: age, sex, cause of visit, blood pressure, arterial blood gas analyses with lactate, glucose, creatinine, ALT, AST, GGT, C-reactive protein, drugs, history of heart failure, pulmonary disease or obesity, and destination. Statistical analysis was performed using SPSS 21.0.
Results
Total of 221 patients, 83 (37.6%) non-diabetics and 138 (62.4%) diabetics, of these 65 (47.1%) treated with metformin. Mean serum lactate and prevalence of hyperlactacidemia were significantly higher in T2D patients relatively to control group (2.1 ± 0.1 vs 1.1 ± 0.1, $P < 0.01$ and 39.1 vs 3.6%, $P < 0.01$ respectively), and in T2D patients under metformin compared to diabetics without this drug (2.7 ± 0.2 vs 1.6 ± 0.1, $P < 0.01$ and 56.9 vs 23.3%, $P < 0.01$ respectively). T2D patients on metformin presented a 25-fold increased risk of hyperlactacidemia (OR = 25.10, $P < 0.05$). Creatinine level was the only independent predictive factor for lactate increased concentrations ($\beta = 1.33$, $P < 0.05$). Patients with hyperlactacidemia had 4.4 higher odds of being hospitalized or die (OR = 4.37, $P < 0.05$).
Conclusions
T2D patients, particularly those under metformin, presented significantly higher serum lactate and prevalence of hyperlactacidemia. Creatinine level was the only independent predictive factor for lactate increased concentrations. Hyperlactacidemia implies a worse prognosis and must be discarded in T2D patients observed in ER.

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P421

Type 1 diabetes and pregnancy: importance of glycemic control in maternal and perinatal outcomes

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Introduction
Type one diabetes is estimated to account for 1% of the pregnancies complicated by diabetes, associated with an increased risk of maternal and perinatal morbidity. The multidisciplinary follow up of the diabetic women can contribute to an improvement of the glycemic control, minimizing the obstetric and perinatal complications.
Aims
To characterize pregnant women with type one diabetes followed in our Department's Endocrinology/Obstetrics Outpatient Clinic. To correlate their glycemic control with maternal and perinatal complications.

Methods
Retrospective analysis of clinical, analytical and therapeutic data of 158 pregnant women with type one diabetes followed between 1995 and 2012. Assessment of maternal complications, type of delivery and perinatal morbidity according to their glycemic control, using SPSS 21.0®.
Results
Sample of 158 women, mean age 28.7±5.3years, with type one diabetes for 11.8±7.2years, followed from 9.8±5.4weeks' gestation, mean A1c in the 1st Trimester=7.7±1.5%, 2ndT=6.5±0.9% and 3rdT=6.6±0.9%.
Regarding the maternal outcomes: microvascular complications were worsened in 19 women (12.1%), only two episodes of diabetic ketoacidosis (1.3%). Preterm delivery in 40 women (25.3%), pregnancy induced hypertension in 17 (10.8%), preeclampsia in 20 (12.7%).
Perinatal morbidity was significantly higher in women with A1c>7% in the 1st Trimester (39.4% Vs 22.5%;$P=0.041$), 2ndT(57% Vs 27.4%;$P=0.007$) and 3rdT(51.4% Vs 29.1%;$P=0.033$), when compared with women with adequate glycemic control (A1c=6-7%). Congenital malformations were significantly more prevalent in women with A1c>7% in the 1stT(9% Vs 0%;$P=0.003$), 2ndT(17.9% Vs 1.6%;$P=0.004$) and in the 3rdT(11.4% Vs 1.8%;$P=0.048$). There were two cases of stillbirths (1.3%) correlated with A1c>7% in the 3rdT ($P=0.035$).
Concerning the type of delivery, the rate of caesarean section was high (63.8%) and significantly superior in the women with excessive gestacional weight gain ($P<0.001$).
Conclusion
The prevalence of perinatal morbidity was significantly correlated with the glycemic control in all three trimesters, stressing the importance of a tight and permanent follow up of the pregnant women with type one diabetes.

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P422

Assessment of glycemic control in patients with T1DM and depression

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Aim
Is to determine the frequency of hypoglycemic states in patients with T1DM with and without depression.
Materials and Methods
patients with T1DM (57 men and 51 women) aged 41.15 years (34.65, 46.66) were examined, with the experience of diabetes about 11.32 years (2.80, 11.90). Depression was determined according to the scale HADS. Assessment of glycemia was carried out with the help of daily monitoring system of glucose in intercellular fluid (CGMS).
Results
The patients were divided into two groups: 1st – patients with T1DM with depression ($n=47$), 2nd – patients with T1DM without depression ($n=61$). The HbA1C level in the first group was 9.75% (8.70, 10.45) vs 8.15% (7.50, 9.45) in the second group (?(0.05).
The level of minimum glycemia in patients of the first group was 2.45 (2.20, 3.45) mmol/l vs 4.4 0 (3.50, 5.70) mmol/l in the comparison group ($P<0.05$);
– the duration of hypoglycemia in the first group was 2% (0, 8) vs 0% (0, 0) in the comparison group ($P<0.05$);
– 35 hypoglycemic states (79.54%) were recorded in the first group versus 18 (29.5%) in the second group (P(0.05);
– there were 26 (59.1%) cases of latent hypoglycemia in the first group versus 11 (18%) in the second group (P(0.05).
The relationship between increased levels of NbA1C and depression was found (RC=1.50; $P=0.002$; 95% CI 1.15–1.94). A similar dependence was traced when assessing the frequency of episodes of latent hypoglycemia and depression level HADS (RC=1.12; $P=0.0024$; 95% CI 1.02–1.24).
Conclusion
The patients with T1DM and depression had a significantly higher level of HbA1C with a lower level of minimum glycemia and a long duration of hypoglycemia. Frequent hypoglycemic episodes, including latent hypoglycemia, may be the reason of decompensation.

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P423

The peculiarities of atherosclerotic coronary arteries lesion in patients with coronary artery disease and type 2 diabetes mellitus

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Purposes
To assess the peculiarities of atherosclerotic coronary arteries (CA) lesion in patients with coronary artery disease (CAD) and type two diabetes mellitus (T2DM).
Materials and methods
We provide a retrospective analysis of 90 cases of patients with CAD (54 males, aged 60,5±4,7 years). Baseline characteristics of patients included history of CAD (7,2±2,3 years), T2DM (4,7±0,5 years). All patients were divided into two groups: 1st group – patients with concomitant T2DM ($n=30$), 2nd group ($n=60$) – patients without concomitant T2DM. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL), very LDL (VLDL), triglycerides (TG), high-density lipoprotein cholesterol (HDL), fasting blood glucose and level of HbA1c were determined. The presence and extent of CA occlusion were performed using coronary angiography.
Results
Among 1st group of patients in 73% cases an atherosclerotic lesion of two and three CA has been registered, which localized in the middle and distal segments of CA, in the 2nd group in 67% cases an atherosclerotic lesion of one CA has been registered ($P<0,05$). The degree of occlusion of the CA was significantly higher in the 1st group in which rates of TC, LDL cholesterol, TG, HbA1c were greater. The level of HbA1c positively correlated with the degree of CA occlusion ($P=0,38$, $P<0,05$). We also evaluated positive correlation between the degree of CA occlusion and the level of LDL cholesterol ($r=0,56$, $P<0,05$), and TG levels ($r=0,49$, $P<0,05$).
Conclusions
Among patients with CAD and T2DM a diffuse and widespread coronary arteries injuries are mostly registered, as well as multivessel lesions which are mainly localized in the middle and distal segments of CA. These changes were associated with significantly higher levels of LDL cholesterol, TG and lower HDL cholesterol, and parameters of carbohydrate metabolism.

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P424

The influence of resistin on liver function in patients with diabetes mellitus type 2 and obesity

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There is a need in study of resistin (R) role in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) against the background of metabolic disorders.
Aim
To assess the relationship between R and indexes of enzyme and pigment metabolism in patients (pts) with NAFLD combined with diabetes mellitus (DM) type 2 and obesity (Ob).
Methods
Fifty pts with NAFLD in combination with subcompensated DM (HbA1c 8.3±1.7%) and Ob (BMI≥30 kg/m^2) were examined. The control group included 20 healthy individuals. The R was determined by ELISA method. All patients underwent ultrasound investigation of liver and also liver biopsy.
Results
The level of R in pts was increased (10.0±0.11 ng/ml; $P<0.001$) in comparison with the control (4.87±0.11 ng/ml). The correlation was established between R and aspartate aminotransferase ($r=0.57$; $P<0.05$), alanine aminotransferase ($r=0.49$; $P<0.05$), total bilirubin ($r=0.59$; $P<0.05$), conjugated bilirubin ($r=0.71$; $P<0.05$), alkaline phosphatase ($r=0.82$; $P<0.05$).
Conclusion
The increase of resistin level can negatively affect the pathogenesis of NAFLD, amplifying the processes of cytolysis, cholestasis and fibrogenesis in patients with comorbid DM and Ob.

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P425

Risk factors and outcomes of periferal artery disease in patients with diabetes mellitus

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Objective
To analyse features of peripheral artery disease (PAD) in patients with diabetes mellitus (DM), to determine the consequences of PAD and the risk factors for amputation.
Material and methods
Retrospective study of 925 diabetic patients (58% males, 95.6% with type 2 DM) with PAD treated in Vascular Surgery Centre in Vilnius City University Hospital in 1997–2011 was performed.
Results
Mean age was 67.99 ± 9.47 years; mean duration of having DM was 12.95 ± 9.91 years. 47.8% of patients had diabetic angiopathy, 34.9% – nephropathy, 14.6% – retinopathy and 33.7% – polyneuropathy. On admission to the hospital 56.5% of patients had intermittent claudication or permanent leg pain, 82.5% had foot defects, including gangrene or ulcer. 30% T1DM patients and 43.2% T2DM patients with foot defects were painless.
Three hundered and sixty nine patients underwent amputation: 53.4% fingers' amputations, 29.3% below-knee amputations and 17.3% above-knee amputations. Bypass surgery was performed in 360 (39%) patients; revascularization angioplasty procedure (PTA) had 155 (17%) patients.
An average lengh of stay in hospital was 17.3 ± 10.80 days. Mean length of stay in the amputees group was 18.35 ± 10.39 days and it was by 2.35 days longer than after bypass surgery ($P = 0.012$) and 8.9 days longer than after PTA ($P < 0.0001$). Patients without leg's pain were treated by 1.67 days longer than those who suffered pain ($P = 0.014$).
Previous amputation or bypass surgery predicted new amputation (accordingly odds ratio 3.15 and 1.87), but decreased accordingly 2.17 and 1.49 times odds for bypass surgery.
Conclusions
PAD predominated in male patients, age above 60 years, with T2DM, with duration of diabetes more than 10 years and multiple diabetes complications. 39.9% of the diabetic patients with PAD underwent amputations. Patients with amputation stayed in hospital longer than those with bypass surgery or PTA. Previous amputation and bypass surgery were independent risk factors for limb amputation in diabetes patients.

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P426

Early on evaluation of hearing function with transient evoked otoacoustic emissions (TEOAE) and distortion product otoacoustic emissions (DPOAE) in type 2 diabetic patients without hypertension

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Objective
Altered hearing function in diabetic patients has been found in both pure tone audiometry and otoacoustic emissions (OAEs). We intended to evaluate the cochlear function with both DPOAE and TEOAE in patients with DM without polyneuropathy and hypertension.
Patients and methods
Study group consisted of type 2 diabetic patients and healthy control group consisted of healthy age and sex matched volunteers. Detailed ear examination, pure tone audiometry, TEOAE, and DPOAE were assessed in all volunteers. Electromyography for excluding polyneuropathy was done in patients with DM. Ophthalmoscopic fundus examination was performed to all diabetic patients.
Results
There are not statistically significant difference between groups according to sex, gender and smoking habits ($P > 0.05$). Right ear TEOAE 20 kHz frequency SNR ($P = 0.043$), 40 kHz frequency SNR ($P = 0.018$) and left ear TEOAE 20 kHz frequency SNR ($P = 0.004$) are lower in patients with DM. Although, there are differences in respective kHz values between DM and healthy control according to TEOAE test report, there is not statistical significant difference between groups according to overall TEOAE frequencies (left ear ($P = 0.837$), right ear ($P = 0.442$). There is a statistically significant negative correlation between right ear TEOAE 20 kHz SNR ($P = 0.044$) and serum glucose level. Also there is a negative correlation but statistically non significant between right ear TEOAE 40 kHz SNR and retinopathy ($P = 0.053$). No correlations were found among duration of diabetes, HbA1c values, TEOAEs and DPOAEs.
Conclusion
This is the first study in electromyography test negative, nonalcoholic and normotensive type 2 DM patients for evaluating cochlear function with both TEOAE and DPOAE. Albeit TEOAE measurements at some frequencies were significantly reduced in patients with DM, considering all frequencies meaningful reduction of cochlear function was not observed. This suggests us that in patients with DM, cochlear function was not affected.

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P427

Maternal 75 g OGTT glucose levels as predictive factors for large-for-gestational age newborns in women with gestational diabetes mellitus

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Objective
Our goal was to investigate the effects of glucose levels from 75-g oral glucose tolerance test (OGTT) on large for gestational age (LGA) newborns in women with gestational diabetes mellitus (GDM).
Material and methods
A prospective study was undertaken in Outpatient Department of Clinics for Endocrinology, Diabetes and Metabolic Disorders. One hundred and eighteen pregnant women were prospectively screened for GDM between 24 and 28 weeks of pregnancy.
Results
From 118 pregnancies, 78 (66.1%) women were with GDM, and 40 (33.9%) without GDM. Twenty-one (30.4%) of the neonates in the GDM group were LGA (adjusted weight at or above the 90th percentile). This proportion significantly differ from the proportion (5.5%) for the control group ($P < 0.01$). There were significant correlations between LGA from GDM pregnancies with fasting, and 1-h OGTT plasma glucose levels ($r = 0.46$ and 0.23 respectively, $P < 0.05$). Gestation week of delivery and fasting glucose levels were independent predictors for LGA ($\beta = 0.58$ and $\beta = 0.37$ respectively, $P < 0.001$). Areas under the receiver operator characteristic curve (AUC) were compared for the prediction of LGA. The AUC were: 0.782 (0.685–0.861) for fasting, 0.719 (0.607–0.815) for 1-h, and 0.51 (0.392–0.626) for 2-h OGTT plasma glucose levels.
Conclusion
Fasting and 1-h plasma glucose levels from OGTT may predict LGA babies in GDM pregnancies.
Key Words
gestational diabetes, oral glucose tolerance test (OGTT), large for gestational age.
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P428

The influence of erythropoietin therapy on renal function and proteinuria level in patients with early diabetic nephropathy

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Introduction
Anemia is associated with an increased risk of cardiovascular events and death in patients with diabetic nephropathy (DN). However, there are still a lot of contradictions related to the benefits and risks of antianemic treatment in this group of patients due to variety of non-hematopoietic effects of erythropoietin. The aim of this study was to assess the influence of erythropoietin therapy on renal function and proteinuria in patients with early DN.
Methods
We included 36 patients with type 2 diabetes mellitus and early DN (chronic kidney disease, stages 1–3) complicated with renal anemia, not receiving hemodialysis. The main group (17 patients) received standard doses of erythropoietin-α s.c. and iron medication orally for 16 weeks. The control group (19 patients) received only oral iron medications for the same period of time. We assessed serum creatinine and urea levels, glomerular filtration rate (estimated by Cockraft–Gault formula) and urinary protein concentrations before and after 16 weeks of treatment. The Wilcoxon non-parametric test (W) was used to compare these values in study groups.
Results
Before treatment we found the following mean values in the main and the control group, respectively ($P=0.05$): serum creatinine – 118.7 ± 10.1 and 115.4 ± 8.8 μmol/l, serum urea – 11.2 ± 0.9 and 10.7 ± 0.6 mmol/l, estimated glomerular filtration rate – 65.5 ± 5.2 and 63.1 ± 6.9 ml/min*1.73 m^2, urinary protein concentration – 0.50 ± 0.09 and 0.48 ± 0.10 g/l. After 16 weeks of treatment estimated glomerular filtration rate, creatinine and urea levels have not undergone significant changes ($P>0.1$). At the same time mean urinary protein concentration has significantly decreased in the main group as compared to the control group – 0.29 ± 0.05 and 0.50 ± 0.11 g/l, respectively ($W=2.90$; $P=0.004$).
Conclusion
The results suggest that comprehensive antianemic therapy with erythropoietin and iron medications leads to a reduction of proteinuria level in anemic patients with early stages of DN. This beneficial effect can be taken in account when defining the indications for erythropoietin administration.

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P429

The diabetic hand: a forgotten complication?

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Aim
To investigate the prevalence of the most frequently occurring hand complications in 1000 type 2 diabetes mellitus patients.
Material and methods
patients (mean age 50 ± 9.01 years, 34 women and 24 men) who had type II DM were included as a preliminary in this study. The presence of cheiroarthropathy, Dupuytren's contracture, tinnel sign and tendinitis was assessed. All patients were evaluated also by the Rheumatology Division. Diabetic retinopathy was assessed by direct ophthalmoscopy. Urinary albumin excretion was determined in at least two 24 h urine samples.
Results
The mean diabetic duration was 6.67 ± 5.08 (min: 1 max: 25) years. Dupuytren's contracture was present in 5.2%, cheiroarthropathy in 15.5%, tinnel sign in 15.5% and tendinitis in 6.9%. Retinopathy was present in 10.3% nephropathy in 8.6%. Mean HbA1c was 8.54 ± 1.89. Mean fasting glucose was 178.19 ± 66.03 mg/dl. The mean urinary albumin excretion was 40.79 ± 14.92 mg/day. Mean creatinine clearence was 104.78 ± 33.98 ml/min. The relationship between these complications and patients' age, sex, duration of diabetes and glycaemic control was also analysed. There was positive correlation between age and tendinitis and Dupuytren's contracture ($P=0.024$, $r=297$ and $P=0.020$, $r=305$ respectively). There was positive correlation between diabetic duration and diabetic retinopathy ($P=0.026$, $r=295$). There was positive correlation between diabetic duration and cheiroarthropathy and tendinitis ($P=0.044$, $r=268$ and $P=0.012$, $r=332$ respectively). There was positive correlation between diabetic nephropathy and cheiroarthropathy and tinnel sign ($P=0.00$, $r=547$ and $P=0.003$, $r=377$, respectively). There was positive correlation between urinary albumin excretion and cheiroarthropathy and tinnel sign ($P=0.00$, $r=632$ and $P=0.000$, $r=477$, respectively).
Conclusions
Some musculoskeletal disorders are more prevalent in type 2 diabetes mellitus patients and this may be associated with duration of diabetes. Also, the hand abnormalities were associated with the diabetic complications. Long-term prospective randomised controlled trials on preventing musculoskeletal complications and disability in diabetics are needed.

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P430

Echocardiographic parameters and blood pressure in normo-albuminuric prehypertensive diabetic patients

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Introduction
Elevated blood pressure levels are more frequently observed in diabetic patients, than in the general population. Ambulatory blood pressure monitoring (ABPM) is better correlated with target organ damage from hypertension (HT) than clinic blood pressure readings. The correlation between ABPM and urinary albumin excretion rate (UAER) in diabetes mellitus (DM) has been found to be stronger than the correlation between clinic blood pressure (BP) and UAER.
Aim
To investigate the relation between echocardiographic parameters and 24-h ABPM in normoalbuminuric pre-hypertensive diabetic patients, without clinical evidence of nephropathy or cardiovascular autonomic neuropathy.
Methods
In our study have been enrolled 40 adult patients, 20 of them diagnosed with type 1 DM (T1DM) and 20 others with type 2 DM (T2DM). They categorized as dippers and non-dippers on the basis of 24-h ambulatory blood pressure measurement and their echocardiographic parameters were compared. An oscillometric portable monitor took 24-h BP measurements automatically. A comprehensive echocardiographic evaluation was performed focusing on the left ventricular (LV) dimensions, LV mass index (LVMI), relative wall thickness (RWT), left atrial (LA) dimension and LV ejection fraction.
Results
Of the 20 T1DM pre-hypertensive patients, 9 were categorized as dippers and 11 as non-dippers, while of the 20 T2DM pre-hypertensive patients, 12 were categorized as dippers and 8 as non-dippers. There were no differences between the dippers and the non-dippers T1DM pre-hypertensive patients about the age, gender, body mass index (BMI) and clinical and ABPM for average day-time systolic and diastolic blood pressure levels. According to type 2 diabetic pre-hypertensive patients, there were differences about all above parameters. LV internal diameters, LV septal and posterior wall thicknesses, LV ejection fraction (LVEF) were all similar in both groups. Left atrial diameter and LVMI were found higher in the non-dippers diabetic patients. RWT resulted higher in the non-dippers patients.
Conclusion
Among pre-hypertensive normoalbuminuric diabetic patients evaluated by ABPM, echocardiographic parameters disorders resulted more frequent in T2DM. The most important conclusion of this study was that non-dippers had higher LVMI and left atrial dimensions compared with dippers. This may be a predictor of worse long term cardiovascular outcomes in non-dippers.

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P431

Relationship between endothelin-1 levels in diabetics with and without microangiopathy and control subjects

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Aim
To assess ET1 levels in diabetic patients with and without microangiopathy, and compared with normal subjects, nondiabetic, without underlying vascular pathology.
Introduction
The pathophysiology of diabetic microangiopathy is complex and many important aspects of it still are not fully understood. Endothelial dysfunction is of major importance in the pathogenesis of atherosclerosis and diabetic angiopathy. ET-1 is one of the most potent vasoconstrictors described and has been suggested to be involved in the development of cardiovascular disease.
Subjects and methods
We analyzed a group of 21 diabetic patients, 8 women and 13 men, aged (X ± SD) of 61.3 ± 12.6 years (range 21–78) with an evolution of diabetes in 8.4 ± 9 years (range 1–30). 2 patients had type 1 diabetes and 19 had type 2 diabetes. The 67% was on insulin therapy and 52% had microvascular involvement. The control group were 34 persons, 21 women and 13 healthy men, aged 46 ± 21.1 years (range 24–78 years) without known pathology. Statistical analysis was carried out by SPSS. ET1 was performed by RIA Levels.
Results
ET1 levels in diabetic patients without microvascular disease ($n=10$) were 5.6 ± 3 vs 8.09 ± 2.74 pg/ml ($P=0.026$) in the diabetic group affections of microangiopathy ($n=11$). In the control group ($n=34$) ET1 levels were 3.71 ± 1.87 pg/ml.
Conclusions
– ET1 levels in diabetic patients, predominantly type 2, with microangiopathy appear to be higher than in the group did not affect microvascular disease.
– The absence of microvascular disease raises no ET1 levels in diabetic patients compared to control subjects.
– The production and the plasma levels of ET-1 are elevated in patients with diabetes, and a positive correlation between plasma ET-1 levels and diabetic microangiopathy has been reported, suggesting a potential role of the endothelin system in the pathophysiology of vascular complications in diabetes.
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P432

Diabetic polyneuropathy predictors
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Introduction
Diabetic polyneuropathy is detected in 12.5–14.5% of patients after 5 years of falling ill.
Aim
To detect predictors of diabetic polyneuropathy development in prospective parallel open clinical research.
Methods
From 2010 to 2012, 189 patients were examined (108 men, 81 women, average age – 38 ± 7 years) with DM type 2 (82%) and type 1 (18%), without neurological complaints. We estimated the neurological state, detected vibration sensation, tactile sensibility, pain, temperature sensitivity on legs, conducted electroneuromyography. Two subgroups were defined: i) 48 patients with sensory reply amplitude in n. suralis 10-19 mV; ii) 141 patients with sensory reply amplitude in n. suralis <10 mV. The research was conducted twice with a year interval.
Results
In 12 months in 10 cases of the first subgroup (21%) there were complaints of numbness and pains in legs, reduction of achilles reflexes; 10 patients (21%) – reduction of vibration sensation; 1 patient (5%) – pain sensitivity reduction; 1 patient (5%) – temperature sensitivity reduction. In the second subgroup in 12 months later 58 patients (41%) complained of numbness and pains in legs, achilles reflexes reduced ($P=0.047$); 58 patients (41%) had a reduction of vibration sensation ($P=0.047$); 15 patients (11%) – tactile sensibility reduction ($P=0.025$); 18 patients (13%) – an impaired pain sensitivity ($P=0.048$).
Conclusion
Patients with DM have sensory reply amplitude <10 mV in n. suralis when conducted electroneuromyography which is a predictor of a developing in a year distal sensory diabetic polyneuropathy.
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P433

Endothelial dysfunction in diabetes mellitus
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Objective
To identify the frequency of occurrence of endothelial dysfunction dorsalis pedis arteries in diabetes.
Materials and methods
patients with diabetes mellitus, with no complaints of the legs were examined (aged 40.25 ± 3.12, duration of diabetes 12.50 ± 1.25). Endothelial dysfunction was studied with high-resolution ultrasound (9 MHz) and diagnosed in the absence of growth in artery diameter in response to reactive hyperemia $>10\%$ or at the appearance of paradoxical vasoconstriction.
Results
Endothelial dysfunction was diagnosed in 39 patients (29.3%), while violation macrocirculation was confirmed in 19 patients (14.3%). Among patients with endothelial dysfunction blood flow disturbance in the arteries of the foot was diagnosed in 48.7%. The result of the discriminant analysis showed that increased levels of HbA1c $>7.5\%$, cholesterol >6.5 mmol/l and triglycerides >1.7 mmol/l increase the risk of endothelial dysfunction. The risk of endothelial dysfunction also significantly increases with disease duration >5 years, and if there is hypertension in a case history. Total accuracy of the discriminant model was 82.5%, at $P<0.05$.
Conclusion
Endothelial dysfunction is an early indicator of clinical and instrumental form of angiopathy. The presence of hypertension and the lack of compensation of carbohydrate and lipid metabolism increase the risk of endothelial dysfunction.
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P434

Risk factors and diabetic foot wound classification: ten years of follow-up
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Objective
To outline the risk factors, the epidemiological profile, and the classification of diabetes type two foot ulcer.
Methods
A prospective observational study, including all the people that sought out in a consecutive way, a tertiary diabetic foot service, from February 2002 until September 2012. At admission, the patients were examined and had their ulcers evaluated by only one observer, in accordance with the University of Texas classification.
Results
Were evaluated 973 patients. The average age was 64.6 years ± 12.6 and 52.8% of them were female. The average age of the diagnosis of diabetes was 53.9 years ± 14.2. Arterial hypertension was present in 69.4%, 38% of the patients had ischemia and 74.7% presented infection. When divided according to gender, the average age of the females was 66.4 years ± 12.4 and 62.41 years ± 12.6 ($P<0.01$) for the males. The diagnostic time for the females was 11.3 years and 9.52 years for the males ($P<0.01$). In the classification of the wound, the prevalence of ischemia was 38% and of infection 74.7%. Penetrating to tendon or capsule were 16.9%. Penetrating to bone or joint, were 13.8%.
Conclusions
Even though there were a higher proportion of women, the men were significantly younger and had less sickness time than the women. The prevalence of ischemia

and infection was greater than that reported in current literature (10.7 and 38%, respectively).

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P435

Smoking and progression of diabetic nephropathy in type 1 diabetes mellitus

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Objective
To investigate the association between cigarette smoking and the progression of diabetic nephropathy in type 1 diabetic patients.
Research design and methods
A prospective, follow-up study over 1 year was conducted in a sequential sample of 30 smokers, 30 nonsmokers, and 30 ex-smokers with type 1 diabetes, hypertension, and diabetic nephropathy. Progression of renal disease was defined according to the stage of nephropathy as an increase in proteinuria or serum creatinine or a decrease in the glomerular filtration rate.
Results
Progression of nephropathy was less common in nonsmokers (10%) than in smokers (59%) and patients who had quit smoking (29%), $P > 0.001$. In a stepwise logistic regression analysis, cigarette pack years, 24-h sodium excretion, and GHb were independent predictive factors for the progression of diabetic nephropathy. Because blood pressure (BP) was well controlled in these patients and most values were within a normotensive range, neither standing, sitting, nor supine BP values were associated with progression of nephropathy.
Conclusions
Cigarette smoking represents an important factor associated with progression of nephropathy in treated hypertensive type 1 diabetic patients.

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P436

Association of C-reactive protein and nephropathy in type 1 diabetic patients

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Aim
To determine the relationship between C-reactive protein (CRP) and nephropathy in type 1 diabetic patients.
Methods
A total of 68 patients (24 women and 44 men) with type 1 diabetes aged 46.58 ± 12.23 years were studied. All subjects had BMI < 35 kg/m^2. The levels of CRP, fasting blood glucose (FBG), triglycerides (TG), HbA1c and renal function tests were assessed. Patients were divided into 2 groups: 1 group – patients with nephropathy (patients were classified as normoalbuminuric (albumin excretion rate (AER) < 30 mg/24 h, $n = 14$), microalbuminuric (AER 30–300 mg/24 h, $n = 38$) and proteinuric (AER > 300 mg/24 h, $n = 16$) and 25 healthy subjects as a control.
Results
The duration of diabetes was 14.60 ± 7.81 years. There was significant difference between group 1 and group 2 regarding the level of CRP (4.22 ± 1.74 vs. 1.54 ± 0.31, $P < 0.001$). We found a positive significant correlation between CRP and FBG ($r = 0.65$, $P < 0.05$), CRP and HbA1c ($r = 0.54$, $P < 0.05$) and CRP and TG ($r = 0.30$, $P < 0.005$) in group 1.
The multivariate linear regression analysis showed significant association CRP with AER ($B = 0.53$, $\beta = 0.681$, $P = 0.02$).
Conclusions
The level of CRP is sensitive markers for diabetic nephropathy in type 1 diabetic patients. The further evaluation will be needed to clarify this association.

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P437

Relation of high sensitive CRP and insulin resistance to retinopathy in type 2 diabetes

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Background
Pathophysiology of Diabetic retinopathy is a complex process. The aim of this study was to evaluate the association between insulin resistance, high sensitive CRP level as inflammation markers and diabetic retinopathy.
Methods
In this cross-sectional, 342 patients with type 2 diabetes were selected. The following data were recorded: age, sex, duration of diabetes and type of medical treatment. HbA1c, FBS, HSCRP, lipid profiles and insulin level were measured for all patients. Ophthalmologic examination was performed for all patients by an expert ophthalmologist. Insulin resistance was calculated by HOMA-IR formula. Relation between HSCRP levels and HOMA-IR was evaluated with diabetic retinopathy.
Results
A total of 342 patients (108 male, 234 female) were enrolled. The mean age of patients was 55.05 ± 9.8 years. Prevalence of diabetic retinopathy in our patients was 30.4% (21% non-proliferative retinopathy and 9.35% proliferative retinopathy). This analysis was shown that HbA1C and duration of diabetes are only independent predictive factors for diabetic retinopathy. There was a differences between the serum hsCRP levels of those with and without retinopathy, however this difference was not significant ($P = 0.47$). A significant association was found between diabetic proliferative retinopathy and insulin resistance ($P = < 0.001$).
Conclusion
It seems, apart from known risk factors for diabetic retinopathy, insulin resistance is one of the possible factors for progression of diabetic retinopathy.
Key Words
Retinopathy, High sensitive CRP, Insulin resistance, HOMA-IR, Diabetes, Inflammation

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P438

Assessment of hypoglycemia risk factors in insulin treated patients

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Achieving glycemic targets is crucial in the management of diabetes mellitus. Insulin is the most effective hypoglycemic treatment for diabetes but its use can be associated with a risk of hypoglycemia.
The aim of this study is to specify risk factors of hypoglycemia in insulin-treated patients.
Patients and methods
This transversal study included 69 patients. They were females in 49.4% of cases ($n = 41$). Mean age was 52 years (14 –77). Diabetes was a type 2 diabetes in 65.2% of cases ($n = 45$), a type 1 in 24.6% ($n = 17$) and a secondary diabetes in 10.1% ($n = 7$). Mean duration of diabetes was 13.9 years (2–32). Mean HBA1c was 10.9% (6.7 to 17.7%). Mean insulin dose was 0.79 IU/kg per day (0.14–1.7). All patients had a normal renal function.
Results
Hypoglycemia occurred in 81.2% of patients. Mean frequency of moderate and severe hypoglycemia were respectively 14.8 and 3.5 per month. No correlation was found between gender and type of diabetes on one hand and the occurrence of hypoglycemia on the other hand. No difference in daily insulin doses and number of daily injections was observed between patients with and without hypoglycemia. Lipodystrophies and injections into lipodystrophies were more frequent in patients with hypoglycemia but with no significant difference ($P = 0.08$). Occurrence of hypoglycemia was negatively correlated to education for the technique of insulin injection ($P = 0.086$) and positively correlated to duration of diabetes ($P = 0.012$) and to duration of treatment with insulin ($P < 0.001$).
Conclusion
Hypoglycemia in insulin-treated patients is frequent. Long progression period of diabetes and the lack of education concerning insulin injections seem to be major risk factors of hypoglycemia.

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P439

Admissions of diabetic patients due to hypoglycemia of external cause in a central hospital from 2006 to 2012

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Background
Hypoglycemia is a common complication of Diabetes Mellitus treatment, especially in patients receiving intensive therapy. It can cause severe clinical consequences and is an obstacle to achieve glycemic targets.
Methods
Retrospective study of severe hypoglycemia due to insulin or oral drug therapy that lead to hospitalization in a central hospital. We reviewed the medical records in the last 7 years (2006–2012) for blood glucose levels at admission and pre-hospital care, A1c, diabetes duration, current therapy and comorbidities. There were 242 inpatient hospitalizations of 228 patients. We compared the hypoglycemic rate and the most commonly prescribed class of oral agents and insulin.
Results
The mean age of our patients was 76 (53–93) years, duration of hospitalization was 11 (1–123) days and 3 died in the hospital. In those with hypoglycemia as the main diagnostic ($n=132$), 5.7% had diabetes mellitus type 1 and 94.3% type 2. Of all subjects ($n=242$) 34.5% used insulin and 59.5% oral drugs (78% of them were treated with a sulfonylurea), the remaining 6% were not specified therapy. The mean blood glucose level was 36 mg/dl at pre-hospital care (7–60), at hospital admission 84 mg/dl (7–211) and mean HbA1c was 6.9% (5–13% and 49% had HbA1c below 7%). Most patients had impaired renal function (mean creatininemia 1.6 mg/dl (0.5–6.4)) and macrovascular disease. The hospital admissions rose from 2006 to 2010, and then decreased (assuming 24 admissions in 2006 = 100%: 2007 = 121%, 2008 = 179%, 2009 = 175%, 2010 = 175%, 2011 = 129%, 2012 = 100%).
Discussion and conclusion
The overall admissions due to severe hypoglycemia occurred in older patients. Also frequent was renal function impairement, HbA1c $<7\%$ and treated with insulin or a sulfonylurea. In this decade, overall incidence of admissions due to hypoglycemia in diabetic patients is falling, which match with the change of prescription pattern of oral agents (less sulfonylureas prescribed and the increased use of DPP4 inhibitors).

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P440

Ambulatory blood pressure profiles in type 2 diabetic patients

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Introduction
Arterial hypertension is a major risk factor for micro- and macrovascular complications in type 2 diabetes. Several factors are known to influence blood pressure profile in diabetic patients, such as age, sex, body weight, diabetes duration, insulin dosage, metabolic control, and microalbuminuria. Ambulatory blood pressure monitoring (ABPM) permits the observation of blood pressure throughout day and night. ABPM is better related to end organ damage and cardiovascular morbidity from hypertension than office blood pressure readings. We used our database to study risk factors for abnormal 24-h blood pressure regulation and microalbuminuria in type 2 diabetic patients.
Methods
ABPM was performed in 102 diabetics. Individual least median squares (LMS)-SDSs were calculated for diurnal and nocturnal systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure according to normalized values. The nocturnal blood pressure reduction (dipping) was calculated for SBP as well as DBP.
Results
In diabetics, NBP in particular was significantly elevated (SBP $+0.51$, DBP $+0.58$, MAP $+0.80$ LMS-SD) and dipping of SBP, DBP, and MAP was significantly reduced ($P<0.0001$). Age, diabetes duration, sex BMI, A1C, and insulin dose were related to altered blood pressure profiles; dipping, however, was only affected by age, female sex, and A1C. The presence of microalbuminuria was associated with nocturnal DBP ($P<0.0001$) and diastolic dipping ($P<0.01$).
Conclusions
Our observations revealed a clear link between the quality of metabolic control and altered blood pressure regulation even in patients with short diabetes duration. Nocturnal blood pressure in particular seems to mainly contribute to diabetes complications such as microalbuminuria.

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P441

Studying the effect of hypertriglyceridemia and hyperglycemia on the outcome of pregnancy in diabetics and nondiabetics

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Background
Wide range of biochemical and hematological laboratory values changed during diabetic pregnancy comparable to physiological pregnancies. hyperglycemia is a risk factor for fetal macrosomia. The level of maternal triglycerides have been strongly correlated with excess fetal growth and large for gestational age.
Aim of the study
The aim of this study is to evaluate the effect of maternal hyperglycemia and triglycerides on pregnancy outcome in diabetics and non diabetics. The end points are birth weight, mode of delivery, incubator admission and fetal hyperbilirubinemia.
Patient and methods
We studied the outcome of pregnancy in 60 pregnant women (30 diabetics and 30 non diabetics). Triglyceride above 180 mg\dl after 20 weeks of gestation is considered abnormal.
Results
Pregnant diabetics with higher TG have higher incidence of CS (15 vs 5 cases P value 0.005), more macrosomic babies (6 vs 1 case P value 0.01) and higher incubator admission (12 vs 1 P value 0.0002) than nondiabetics. The combined effect of high TG and hyperglycemia significantly increases the CS, incubator admission and fetal weight in pregnant diabetic than the nondiabetic with normal TG and blood glucose with P values 0.003, 0.001 and 0.001 respectively. Macrosomic babies and CS is more in diabetic with HbA1c $\leq 7\%$ than nondiabetics (P 0.001, 0.003 respectively).

Table 1

Clinical data	Normal TG, no = 15	High TG, no = 15	P
Mode of delivery			
SVD	1 (6.7%)	0 (0%)	
CS	14 (93.3%)	15 (100%)	−0.1
Incubator admission			
Yes	12 (80%)	12 (80%)	0.5
No	3 (20%)	3 (20%)	0.5
Neonatal jaundice			
Yes	3 (20%)	3 (20%)	0.5
No	12 (80%)	12 (80%)	0.5
Macrosomia			
Yes	4 (26.7%)	6 (40%)	0.2
No	11 (73.3%)	9 (60%)	0.2

Conclusion
DM has a great impact on pregnancy outcome whether controlled or not. TG has synergistic effect on pregnancy outcome in presence of DM but has limited effect in the absence of DM.
Key Words
DM, pregnancy outcome, hyperglycemia, hypertriglyceridemia.

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P442

Severe diabetic ketoacidosis in type 2 diabetes mellitus

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Introduction
Severe diabetic ketoacidosis (S-DKA) is a life-threatening condition requiring immediate hospitalization and treatment. Although mostly associated with type one diabetic patients, it is also a common condition in type two diabetes mellitus

(T2DM). Our objective was to determine the main clinical and metabolic alterations associated with S-DKA in T2DM patients.
Methods
We retrospectively analyzed the medical records of 27 consecutive T2DM patients with S-DKA admitted to the Department of Internal Medicine between 2009 and 2012. The following clinical and laboratory data were collected: demographics, current diabetes treatment, previous hospital admissions for DKA, precipitating factors, clinical evolution and mortality. Patients with type one diabetes mellitus were excluded.
Results
Mean age was 40±9 years, 15 (56%) women and 12 (44%) men. Mean glucose was 600±177 mg/dl, pH 6.89±0.06, serum bicarbonate 3.9±1.4 mmol/l, osmolarity 306±16 mmol/l, urinary ketones 69.4±24.5 mmol/l, and anion gap 29.9±4.2. The most common contributing factor was noncompliance with diabetes treatment (48%), followed by infection (37%), stroke (4%), and unknown causes (11%). The chief complaints were dyspnea (33%), altered consciousness (26%), malaise (26%), nausea & vomiting (11%) and abdominal pain (4%). Diabetic syndrome was present in 22% of patients. Tachycardia, tachypnea and hypotension were detected in 52, 93 and 19% respectively. DKA resolved after 17±9 h of treatment, with a mean dose of insulin infusion of 0.14±0.06 U/kg per hour. Seventy-four percent of patients were treated with bicarbonate replacement therapy. There were no deaths nor complications documented in the medical records. Length of hospital stay was 4.9±3.7 days.
Conclusion
S-DKA in T2DM is a common condition that requires medical attention. Although considered life threatening, our results indicate that a proper management can yield satisfactory outcomes without mortality or the development of major complications.

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P443

Disabilities of the arm, shoulder and hand (DASH) questionnaire and diabetic complications: preliminary results

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Aim
DASH questionnaire is a self-administered region-specific outcome instrument developed as a measure of self-rated upper-extremity disability and symptoms. The DASH consists mainly of a 30-item disability/symptom scale. In this study, we aimed to evaluate DASH Questionnaire in 1000 type two diabetes mellitus (DM) patients.
Material and methods
Fifty-eight patients (mean age 50±9.01 years, 34 women and 24 men) who had type II DM were included as a preliminary in this study. The presence of cheiroarthropathy, Dupuytren's contracture, tinnel sign and tendinitis was assessed. Diabetic retinopathy was assessed by direct ophthalmoscopy. Urinary albumin excretion was determined in at least two 24 h urine samples. DASH questionnaire was administered to the diabetic patients. Direct measurements of parameters were performed with a Tanita body composition analyser. The bioimpedance parameters we measured were body fat percentage (%BF), total body fat (TBF) (kg) and BMI.
Results
The mean diabetic duration was 6.67±5.08 years. Dupuytren's contracture was present in 5.2%, cheiroarthropathy in 15.5%, tinnel sign in 15.5% and tendinitis in 6.9%. Retinopathy was present in 10.3%, nephropathy in 8.6%. DASH score was 72.18±33.02. Mean BMI was 31.57±6.29. Mean TBF was 29.02±11.87 kg. Mean %BF was 33.73±10.14. There was positive correlation between DASH score and cheiroarthropathy, tinnel sign and tendinitis ($P=0.008$, $r=350$; $P=0.002$, $r=406$; $P=0.001$, $r=445$, respectively). There was positive correlation between DASH score and BMI and TBF and %BF ($P=0.000$, $r=462$; $P=0.002$, $r=410$; $P=0.002$, $r=407$, respectively). No correlation was found between DASH score and diabetic nephropathy and retinopathy.
Conclusions
DASH Questionnaire is useful instrument for measuring functional disability in upper extremity complaints of diabetes mellitus patients. It should also be taken into consideration in order to increase the quality of life in DM patients.

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P444

Impact of a comprehensive management of glycemic profile, arterial hypertension and dislypidemia on the onset of type 2 diabetes mellitus

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Objective
To analyze the impact of a specialized management on cardiovascular risk at the onset of type 2 diabetes mellitus.
Material and method
Were analyzed 171 patients who visited our diabetes day hospital (DDH) during 2010–2011, studying the following variables: sex, age, BMI, smoking habit, arterial hypertension (*de novo*/previously known), antihypertensive therapy, dislypidemia (*de novo*/previously known), hypolipidemic therapy, antidiabetic therapy and antithrombotic therapy. Furthermore, were measured other variables at the onset and 3 months after diagnosis of type 2 diabetes mellitus, using the paired Student's *t*-test (SPSSv18.0). The cardiovascular risk calculator (UKPDS RISK ENGINE) was also performed initially and after three months of monitoring.
Results
The sample included 121 men (70.8%) and 50 women (29.2%), with an average age of 53.6±12.6 years old. They showed a BMI of 31.6±6.2 kg/m^2. A 40.9% of them were smokers while 14.6% were previously smokers and 44.4% non-smokers. An associated arterial hypertension was present in a 61.4% of patients (32% *de novo* and 68% previously known)and 48% were treated with a single agent, 39% with two agents and 6.4% with three agents. Regarding to hyperlypidemia it was observed in 72.5% (72.5% *de novo*, 28.5% previously known). According to the antidiabetic therapy we obtain the following data: oral monotherapy (17.4%), double oral therapy (31.1%), triple oral therapy (0.6%), basal insulin (9.6%), pre-mixed insulin (11.9%) and basal-bolus (29.3%). A 66% of patients used antiaggregation or anticoagulation (acetylsalicylic acid 91%, oral anticoagulation 4.4%, double antiaggregation 4.5%).

Table 1

	HbA1c	Weight	DBP	TAD	LDLc	TG	HDLc
Initial	10.6±2.4	88.2±19.3	137±19	78±13	126±39	329±54	40±13
Final	6.4±1.1	87.7±17.9	133.6±20	75±11	100±37	140±86	44±14
	$P<0.05$	NS	NS	NS	$P<0.05$	$P<0.05$	$P<0.05$

Table 2

UKPDS risk engine		CHD	Fatal CHD	Stroke	Fatal stroke
Men	Initial	26.4	14.6	3.5	0.4
	Final	12.0	4.8	3.3	0.4
Women	Initial	17.0	11.1	4.3	0.6
	Final	7.9	4.1	3.9	0.5

Conclusions
It is essential the development of functional units to perform an education for health combined with a comprehensive and an intensive management of the associated diseases and risk factors present at the onset of type 2 diabetes mellitus, regarding to the determining clinical benefit which is obtained.

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P445

Monoballism associated with newly onset ketotic Hyperglycemia

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Movement disorders as the initial symptoms of diabetes mellitus are rare. Here, we describe one of these rare manifestations of primary diabetes: a case of newly diagnosed diabetes mellitus in an old age female patient with transient monoballismus during an episode of ketotic hyperglycemia. Ballism can be rapidly controlled by normalization of glycemia. Our patient had monoballism confined to her upper extremity. To our knowledge, this is the first report

describing monoballism in a patient with ketotic hyperglycemia. She had a rapid symptomatic remission after correction of the hyperglycemia.
Hemichorea-hemiballismus (HC-HB) constitute a neurological syndrome characterized by violent proximal involuntary movements on one side of the body, involving mainly the upper extremity (1). Focal epilepsy, transient chorea or ballism provoked by an episode of nonketotic hyperglycemia (NKH) in adults with type 2 diabetes (1-8), and ketotic hyperglycemia in children with type I diabetes mellitus have been reported (9). Nonketotic hyperglycemia occurs more often in women (1,3,6) and usually is associated with very high blood glucose (3). In these cases, the seizures (7) as well as the choreiform movements have resolved within days to a few weeks after normalization of blood glucose and hence, reversible metabolic derangements within the basal ganglia have often been assumed (1-4,8,9). Most of the cases have MRI changes in the putamen with high signal intensity on T1-weighted images and variable signal characteristics ranging from hyper-, to iso-, to hypo-intensity on T2-weighted images (1-3,6,8).
Movement disorders as the initial symptoms of diabetes mellitus are rare (5,8). Here, we describe one of these rare manifestations of primary diabetes: a case of newly diagnosed diabetes mellitus in an old age female patient with transient monoballismus during an episode of ketotic hyperglycemia.

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P446

Comparison of Irbesartan and combination with *Nigella sativa* oil in experimental diabetic nephropathy

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Introduction
Diabetic nephropathy is the leading cause of chronic renal failure worldwide. Related to the increased glucose and its metabolites, activation of renin–angiotensin–aldosterone system, and increased oxidative stress are important in renal deterioration. The studies indicate that suppression of renin–angiotensin–aldosterone system slow down diabetic nephropathy; *Nigella sativa* decreases blood glucose and oxidative stress. Were viewed the literature about clinical and experimental studies of diabetic nephropathy but could not find a study involving combination therapy of anti-oxidant medication and angiotensin receptor blockers. In our study; we aimed to study the effects of angiotensin tip II receptor blocker irbesartan at a dose without blood pressure lowering effect and the combination of irbesartan and *Nigella sativa* on experimental nephropathy.
Design
At the end of the study, the renal functions, metabolic markers were assessed at sacrified rats after a 24 h urine collection. Collagen and inducible nitricoxide synthase deposits were evaluated in renal cortex specimens. The data of healthy, sick control and treatment groups were compared. The treatment groups were 5 mg/kg per day irbesartan; 5 mg/kg per day irbesartan and *Nigella sativa* oil 0.2 ml/day per body weight.
Results
In their besartan treatment group, improvement in renal function was observed compared to the sick control group because of significant decrease in glomerular filtration rate, urine output, urinary excreted albümin. The findings of treatment with irbesartan in combination with Nigella was more beneficial because of the decrement in blood glucose and urinary glucose excretion and increment in both renal and rat weights. In addition, there was a significant improvement in renal histopathology of the rats given irbesartan in combination with *Nigella sativa*.
Conclusion
Our findings suggested that in addition to tight glycemic control, suppression of renin–angiotensin–aldosterone system is crucial and administration of *Nigella sativa* oil improve this renoprotective effect for preventing renal deterioration due to diabetes mellitus.

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P447

Association lipids with albuminuria in patients with type 1 diabetes

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Aim
To determine the relationship between serum lipids with albuminuria in patients with type one diabetes.

Methods
A total of 150 diabetic patients both sexes aged 41.58 ± 11.33 years were studied. The levels of total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C), HbA_{1c} and renal function tests were assessed. Patients were classified as normo-albuminuric (albumin excretion rate <30 mg/24 h, $n=38$), microalbuminuric (albumin excretion rate 30-300 mg/24 h, n = 79) and proteinuric (albumin excretion rate >300 mg/24 h, $n=33$).
Results
The duration of diabetes was 15.70 ± 8.81 years. The level of TC was significantly highest in proteinuric (6.90 ± 0.36 mmol/l), followed by microalbuminuric (5.38 ± 1.29 mmol/l) and followed by normoalbuminuric (3.22 ± 0.66 mmol/l), ($P=0.002$, $P=0.0006$, respectively).
Patients with proteinuria had significantly higher level of LDL-C compared to the patients with normoalbuminuria (4.14 ± 0.95 vs 1.71 ± 0.47 mmol/l, $P=0.001$). Patients with microalbuminuria had significantly higher level of LDL-C compared to the patients with normoalbuminuria (3.48 ± 1.19 vs 1.71 ± 0.47 mmol/l, $P=0.015$), as well.
The level of HbA1c in normoalbumiuric patients was significantly lower than in microalbuminuric (7.83 ± 1.12 vs 10.40 ± 1.59%. $P=0.004$).
There were no significant differences in levels of TG, HDL-C, VLDL-C between patients with normoalbuminuria, microalbuminuria and proteinuria.
Conclusion
We showed that higher levels of LDL-cholesterol and TG were associated with microalbuminuria and proteinuria in patients with type 1 diabetes. Lowering atherogenic lipids may retard nephropathy progression in these patients.

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P448

Characteristics of the patients treated at the onset of type 2 diabetes mellitus in our Diabetes Day Hospital (DDH) during 2010–2011

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Objective
To analyze the demographic and clinical characteristics of the patients treated at the onset of type two diabetes mellitus in our DDH.
Material and method
During 2010–2011 were analyzed 171 patients treated in our DDH, studying the following variables: sex, age, BMI, waist circumference, family diabetes history, microvascular and macrovascular long-term complications, smoking habit, rate of associated hypertension (*de novo*/ previously known), rate of dyslipidemia (*de novo*/ previously known), systolic blood pressure (SBP), dyastolic blood pressure (DBP), LDLc, triglycerides(TG), HDLc, HbA1c. According to the HbA1c level the treatment was analyzed, using the paired *T*-student test to compare basal HbA1c and after 3 months of monitoring (SPSSv18.0).
Results
121 men (70.8%) and 50 women (29.2%), with an average age of 53.6 ± 12.6 year old were treated in our DDH. They showed a BMI of 31.6 ± 6.2 kg/m2, with a waist circumference of 107.4 ± 13.0 cm. It was described a familial history of 68.4% of DM. At disease onset were observed 22.3% of macrovascular complications and 18.3% of microvascular complications. Smoking habit was identified in 40.9% of patients while previously smokers were 14.6% and non-smokers a 44.4% of them. Associated arterial hypertension was described in a 61.4% of the sample (32% *de novo* and 68% previously known) and associated dyslipidemia in 72.5% (72.5% *de novo*, 28.5% previously known). Other figures included an average SBP of 136.7 ± 21 mmHg, DBP of 78.3 ± 13.8 mmHg, LDLc levels of 120 ± 41.8 mg/dl, HDLc levels of 41.7 ± 13.8 mmHg and TG levels of 290 ± 49.4 mmHg. Clasifying the patients depending on the antidiabetic therapy initiated we obtained the following data: Oral antidiabetic monotherapy ($n=33$, HbA1c 8.6%), double oral antidiabetic therapy ($n=52$, HbA1c 9.7%), triple oral antidiabetic therapy ($n=1$, HbA1c 9.2%), basal insulin ($n=16$, HbA1c 10.7), pre-mixed insulin ($n=20$, HbA1c 11.7%) and basal-bolus ($n=49$, HbA1c 12.1%). Basal HbA1c level was 10.6 ± 2.4% and after 3 months of monitoring 6.4 ± 1.1%, obtaining a −4.2% difference, clinically relevant and statistically significant.
Conclusions
Patients at the onset of type 2 diabetes mellitus have a severe cardiovascular risk. It requires a comprehensive treatment and an educative effort in order to optimize the therapy and minimize the existing risk.

It is essential the development of functional units for a comprehensive and an intensive management of the different associated diseases and present risk factors.

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P449

Lipid profile in type 1 diabetic patients in Kragujevac

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Introduction
The prevalence of hyperlipidemia in type 1 diabetics is 30 to 90%. High total cholesterol, LDL and triglyceride serum level is independent risk factor for cardiovascular disease. High prevalence of malignant atherogenic profile is alarming in type 1 diabetics (high triglyceride, low HDL). It is very often that dislipidemia is associated with diabetes and obesity, as well as hypertension, that pointed to inherited disorders.
The aim of this study is to analyse lipid profile in patients with type 1 diabetes in Kragujevac depending on gender.
Methods
This study included all patients registered in Primary Care with type 1 diabetes mellitus. We determinated lipid profile: total cholesterol (tChol), LDL, HDL and tryglicerides (TAG). Patients were divided according to gender.
Results
We registered 206 patients with type 1 diabetes in Kragujevac (107 men and 99 women). Anamnesticly, previous lipid disorder had 8.2% patients. According to National Guideline Clinical Practice criteria: 55.5% has increased tChol level, 42.1% increased LDL, 29.1% increased TAG i % 33.3 decreased HDL cholesterol level. There is statisticaly significance in LDL (2.55 ± 0.75 vs 3.0 ± 1.18 mM, men vs women, $P=0.09$), but there is no significance in average tChol level (4.73 ± 1.07 vs 5.01 ± 1.39 mM, men vs women, $P=0.379$), TAG (1.4 ± 0.98 vs 1.41 ± 0.89 mM, $P=0.94$) and HDL (1.39 ± 0.39 vs 1.42 ± 0.38 mM, $P=0.808$).
Conclusion
There is some patients who know about lipid disorders, but the prevalence is high. There is statistically significantly higher LDL in women. All these results showed not satisfied liporegulation in type 1 diabetic patients.

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P450

Association of type II diabetes mellitus with hepatocelular carcinoma occurrence. A case control study from Western Nepal

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Objective
To assess the association of type II DM with hepatocelular carcinoma occurrence.
Materials and methods
It was a case control study carried out using data retrieved from the register maintained in the Department of Biochemistry of Manipal College of Medical Sciences, Pokhara, Nepal between 1st January, 2012 and 31st August, 2012. The variables collected were age, gender, HbA1c. All these biochemical parameters were analyzed in the Central Laboratory of our hospital by standard and validated methods. The One way ANOVA was used to examine the statistical significant difference between groups. *Post hoc* test LSD used for the comparison of means of case groups. Odds ratios (OR) were calculated using simple logistic-regression analysis.
Results
The etiological factor for 200 patients of HCC was HBV, HCV, alcohol and cryptogenic. The highest age group belongs to etiological category of HCV with mean of 71.90 ± 3.6 (CI 69.28, 74.52) years and lowest age group belongs to etiological category of HBV with mean of 61.70 ± 5.3 (CI 57.88, 65.52) years. The main imperative basis of HCC in present study was HCV (39.5%) and second most significant cause of HCC was alcohol (26%). Glycated haemoglobin was more in males HCC (7.9%) as compared to females (7.3%). The percentage of type II diabetes mellitus was more in HCC patients when compared to controls. This difference was statistically significant with an odd ratio of 4.63 ($P< 0.001$).
Conclusion
Type II DM influences incidence, risk of recurrence, overall survival, and treatment-related complications in HCC patients.

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P451

HbA1c levels did not correlate with SF-36 and DQOL scores in patients with poorly controlled type 2 diabetes

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Aim
To evaluate the relationship between glycemic control and quality of life parameters in patients with poorly controlled type 2 diabetes.
Methods
Fifty patients with poorly controlled (HbA1c 8–12%) type 2 diabetes included the study and were answered DQOL and SF-36 questionnaires to an assistant in internal medicine. HbA1c levels, age, gender, duration of diabetes, height, weight and waist–hip ratio measurements recorded at the same time.
Results
Mean age of the patients was 55.5 ± 7.5 (26 erkek/24 female) and mean duration of diabetes was 7.02 ± 2.8. Body mass index (BMI) and waist–hip ratio (W/H) means were found 29.0 ± 4.4 and 0.93 ± 0.06. DQOL and SF-36 scores were found 98.5 and 89.2, mean HbA1c was 1.2 ± 9.5% in all patients. HbA1c levels did not correlate with DQOL and SF36 scores ($R=0.06$ (DQOL), -0.19 (SF36)) when Pearson's correlation analysis performed. There were not correlations between quality of life scores and height, weight, BMI, waist–hip ratio values. Overall, the percentages values of quality of life were lower in the patients. When patients divided into two groups with HbA1c values (<9% and above), mean SF36 and DQOL scores were 86.8 and 96.4 in higher A1c group. In the low A1c group, mean SF-36 score and DQOL score was found 92.9 and 101.6. There was no significant difference between the two groups in terms of quality of life.
Conlusion
In our study, diabetes-related complications, education and social status of patients has not been evaluated. Only the relationship between quality of life measures with glycemic control and the values of physical parameters were examined. In our conclusion, quality of life values are generally lower in patients with poorly controlled diabetes and does not correlate with levels of glycemic control.

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P452

Diabetic ketoacidosis in hospitalized patients with hypertriglyceridemia induced pancreatitis. Case control study

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Introduction
Hypertriglyceridemia induced pancreatitis (HIP) is an uncommon condition accounting for 1–4% of cases of acute pancreatitis, mostly associated with poor glycemic control in diabetic patients. Diabetic ketoacidosis (DKA) may complicate the clinical course of HIP; however, few studies have documented this association. Our objective was to identify clinical and demographic differences between HIP patients with co-existing DKA compared to those without DKA.
Methods
Seven consecutive patients with DKA and HIP were compared with 7 age and gender paired HIP controls. We analyzed risk factors for the development of DKA, compared severity of illness, hospital length of stay, diet indications, duration of insulin treatment, and biochemical markers of pancreatitis. Statistical significance was considered if $P<0.05$.
Results
DKA was associated with more severe HIP compared to non-DKA patients, with Ranson's prognostic criteria 4 ± 2 vs 1 ± 1 ($P=0.009$) and APACHE II 9 ± 3 vs 4 ± 2 ($P=0.004$). There were no differences in previous diagnosis of diabetes mellitus (85 vs 71%, $P=1.0$), length of stay (8 ± 3 vs 7 ± 2 days, $P=0.24$), fasting duration (4 ± 2 vs 4 ± 1 days, $P=0.47$), or duration of treatment with insulin infusion (6 ± 4 vs 3 ± 1 days, $P=0.12$). Serum amylase, leukocyte levels and triglycerides did not differ in both groups ($P=0.96$, 0.94 and 0.80). Insulin

dose during infusion treatment (0.09±0.04 vs 0.04±0.02 U/kg per hour, $P=0.012$) and discharge insulin dose (74±32 vs 33±23 U, $P=0.02$) were higher in DKA compared to non-DKA patients.
Conclusion
There were no statistical risk factors associated with the development of DKA in HIP. Even though DKA is associated with more severe HIP, no differences were observed in: length of stay, fasting duration, insulin infusion time, biochemical markers. As expected, DKA is associated with higher insulin dose during insulin infusion time as well as at discharge.

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P453

Deficiency of vitamin D3 in patients with diabetic retinopathy

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Diabetic retinopathy (DR), is the cause of population invalidization which is diagnosed in 30–90% of patients.
Purpose - to examine vitamin D levels in patients with diabetic retinopathy.
Materials and methods
Under our supervision, there were 48 patients (93 eyes). Among examined 26 patients had nonproliferative form DR and 19 – proliferative DR.
Results
Among the patients with type 1 diabetes 23 (52%) patients had a marked deficiency of vitamin D3. And in 48% was determined by hypovitaminosis. In type 2 diabetes in 25 (59%) patients experienced a deficiency of vitamin D3, and in 41% – hypovitaminosis. In patients defined different levels of vitamin D3, depending on the severity of diabetic retinopathy. Thus the presence of proliferative diabetic retinopathy observed moderate degree of vitamin D3 deficiency (37.5±6.1). For patients with nonproliferative DR are characterized by the presence of hypovitaminosis 25(OH)D (63.9±6.6).
Conclusion
For patients with the DR in type 1 diabetes observed average severity of vitamin D3 deficiency, and in type 2 diabetes – severe.

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P454

A poorly controlled type 2 diabetes complicated by an episode of severe hypertriglyceridemia-induced pancreatitis

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Introduction
Although it is recognised that diabetic ketoacidosis may present with abdominal pain, it remains important to exclude an underlying acute pancreatitis which may be masked due to ambiguous presentation.
Case
A 23-year old woman with a history of type 2 diabetes and non-compliance for her oral antidiabetic medication presented to the emergency department with abdominal epigastric pain and nausea starting a few hours before admission. Laboratory examination revealed a mild ketoacidosis while an abdominal CT scan performed the following day demonstrated a severe acute pancreatitis of the body and tail (Balthazar Grade E) despite normal amylase serum levels upon admission, the absence of inflammatory signs and the presence of only mild clinical symptoms. The presence of a lactescent serum was the clue to an extremely high triglyceride level (>10 000 mg/dl) causing the pancreatitis. The hypertriglyceridemia itself was attributed mainly to the diabetic ketoacidosis. There was no family history of hypertriglyceridemia. Treatment with i.v. insulin and hydration successfully resolved the ketoacidosis and hypertriglyceridemia and reversed the episode of acute pancreatitis.
Conclusion
The triad consisting of diabetic ketoacidosis, hypertriglyceridemia and acute pancreatitis is an unusual presentation of poorly controlled diabetes which can occur in type 1 as well as type 2 diabetic adults and children. With this unusual case we emphasize the need to perform an abdominal CT scan in case of persistent abdominal pain in ketoacidosis, especially in the presence of an hyperlipidaemic serum, which can cause falsely low or normal amylase and lipase levels despite an acute pancreatitis.

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P455

Self care practice among diabetic patients in Kathmandu

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Diabetes is expected to rise from 177 million today to 370 million in 2030. Self care is a crucial to keep the disease under control. Appropriate self care practice can keep the disease under control.
The aim of the study was to find out the self care practice among diabetic patients. This is a simple descriptive cross-sectional study was done in Metropolyclinic Kathmandu, Nepal. A total of 50 respondents, who met eligible criteria were purposively sampled and directly interviewed. χ^2 test was used to see the association. Self care practice of the respondents of this study population was examined using the SPSS version 16.0.
Self care practice among the respondents was not satisfactory. Unsatisfactory self care practice was more than half (56.75%) among adequate knowledge respondents. Among respondents having inadequate knowledge, majority (92.31%) had unsatisfactory practice. Majority (74%) had adequate knowledge and satisfactory self care practice was done by only 34%.
Thus it is concluded that satisfactory self care practice was inadequate. There was significant relationship between self care practice and knowledge regarding diabetes among respondents. Thus the study also concluded that adequate knowledge on Diabetes should be provided to the patients for satisfactory self care practice.
Key Words
Self care practice, knowledge, diabetes.

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P456

Newly diagnosed diabetic patient presenting with crural infection

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Introduction
The incidence of diabetic foot in diabetics is 5-10%. We herein aimed to share a formerly undiagnosed diabetic patient presenting with crural infection.
Case
A 48-year-old male patient, without a formerly known disease, presented to a local medical center due to spontaneously progressing ulceration on left leg. The ulcers did not improve with antibiotics in seven days and afterwards he was referred to our hospital. At admission, his plasma glucose was found high and he was hospitalized in endocrinology department with a diagnosis of new onset diabetes and diabetic foot infection. On physical examination, two distinct ulcerations with purulent discharge and necrotic component were noted at left leg; one in the calf and the other superior to medial malleole, each with a diameter of 10 cm with a Wagner classification of 3. His laboratory findings at admission and on follow-up are given in Table. Intensive insulin treatment and antibio therapy were started. Magnetic resonance angiography revealed stenosis in the crural arteries bilaterally and antiaggregant, anticoagulant, vasodilator and statin therapy were added. His clinical signs of infection including fever persisted despite the antibiotic therapy. The ultrasonographic and MRI evaluation of the ulceration site demonstrated abscess formation beneath those ulcers. Surgical debridement and vacuum associated closure were applied respectively. Tissue grefting was applied after the local infection subsided completely. The patient was discharged after clinical stabilization was obtained.

Table 1 Admission and post treatment laboratory

	Admission	Posttreatment
CRP (mg/l)	284.8	15.8
Sedimentation (mm/h)	110	28
White bloodcell	15 000	6600
Neutrophil	13 600 (90.6%)	3500 (53.7%)
FPG (mg/dl)	415	142
Creatinine (mg/dl)	0.66	0.73
HbA1c (%)	13.1	5.2

Conclusion
Diabetic foot infection is probably the most preventable complication of diabetes, with considerable risks of morbidity and mortality. Early intervention for these ulcers and metabolic problems is critical for adequate treatment success. Therefore, one should keep in mind that diabetes may be the underlying cause or the aggrevating factor of a non healing foot ulcer and should evaluate these patients for presence of diabetes.

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P457

Relation between psychiatric symptoms and diabetic complications: preliminary results

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Aim
In this study, we aimed to assess psychiatric symptoms in 1000 type 2 diabetes mellitus (DM) patients.
Material and methods
Fifty-eight patients (mean age 50 ± 9.01 years, 34 women and 24 men) who had type II DM were included as a preliminary in this study. The presence of cheiroarthropathy, Dupuytren's contracture, tinnel sign and tendinitis was assessed. Diabetic retinopathy was assessed by direct ophthalmoscopy. Urinary albumin excretion was determined in at least two 24 h urine samples. Beck's Depression Inventory (BDI) and Beck's Anxiety Inventory (BAI) were administered.
Results
The mean diabetic duration was 6.67 ± 5.08 years. Dupuytren's contracture was present in 5.2%, cheiroarthropathy in 15.5%, tinnel sign in 15.5% and tendinitis in 6.9%. Retinopathy was present in 10.3%, nephropathy in 8.6%. BDI score was 14.14 ± 10.79 and BAI score was 18.05 ± 16.94. There was positive correlation between BDI score and diabetic nephropathy ($P=0.001$, $r=441$). Also there was positive correlation between BDI score and cheiroarthropathy and tinnel sign ($P=0.039$, $r=276$; $P=0.017$, $r=317$, respectively). Positive correlation between BAI score and diabetic nephropathy was detected ($P=0.000$, $r=476$). There was positive correlation between BAI score and cheiroarthropathy and tinnel sign ($P=0.005$, $r=366$; $P=0.010$, $r=343$, respectively). The suggested BDI cutoff of ≥ 17 had 81% sensitivity and 79% specificity and classified as clinically depressed. In our study BDI score ≥ 17 was 34.5%. BAI score ≥ 17 was classifed as moderate and serious anxious. In our study, BAI score ≥ 17 was 37.9%.
Conclusions
Psychiatric symptoms, especially depression and anxiety, are widely seen in patients with diabetes mellitus. Quality of life and disability are correlated with depression and anxiety levels. Therefore, in addition to the recent management of DM, psychiatric symptoms such as depressed mood and anxiety should also be taken into consideration in order to increase the quality of life in DM patients.

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P458

Pelvic pain in a type 2 diabetes patient

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Objective
To present a patient with long standing type 2 diabetes complaining of chronic pelvic pain, due to an uncommon cause of bilateral vas calcification.
Methods
Clinical, laboratory and radiographic data are reported on a 62-year-old diabetic presenting with chronic pelvic pain
Results
A 62-year-old man with a history of 17 years of diabetes presented with chronic dull aching, non radiating pain in the pelvis and in the region of sacal sulcus below 5th lumbar vertebra. There was no history of fever with chills and sweats, dysuria, urgency, frequency of urination. Pain was not aggravated during intercourse. There was no past history of sexually transmitted disease, frequent and extramarital sexual encounters, chronic kidney disease. Complete blood picture and routine urine examination did not reveal any evidence of infection or proteinuria. Fasting and post prandial blood sugars were 104 and 136 mg/dl with Hba1C at 6.7%. Other blood parameters including lipid profile, renal and liver function tests, serum calcium, phosphorous were all within normal limits. X ray showing anteroposterior view of pelvis revealed bilateral serpentine structures with symmetric and regular vas deferens calcification involving vas calcification.
Discussion
The causes of bilateral vas calcification include degenerative changes due to ageing, diabetes mellitus, end stage renal disease with secondary hyperparathyroidism. They give rise to regular calcifications within the muscular components of the vas with preservation of luminal patency. Causes of unilateral vas calcification include inflammatory conditions like tuberculosis, gonorrhea, syphilis, schistosomiasis, and chronic non-specific urinary tract infections. The calcifications are intraluminal and irregular leading to partial or complete occlusion of the lumen. Vasa differentia may calcify after relatively short duration of diabetes if the disease starts after the age of 40, whereas if the disease occurs before the age of 40, it has usually been present for at least 15 years before calcification is noted. Diabetes accelerates the process of senescent calcification of the vas deferens by augmented expression of several bone-associated proteins (e.g. osteopontin, bone sialoprotein, alkaline phosphatase, type 1 collagen, osteocalcin) that facilitate or regulate the calcification process. In addition uremic serum upregulates osteoblast transcription factor Cbfa 1 and osteopontin expression. Diabetic patients with vasal wall calcification may also develop failure of emission, where no sperm reach the posterior urethra due to aperistalsis of the vas deferens.
Conclusion
Type 2 diabetic subjects with long standing pelvic pain and without any elicitable cause should be evaluated for this uncommon etiology of vas calcification.

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Marking out group of the increased risk of disorder of the mineralization of the osseous tissue at patients with diabetes

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The aim of this research was foot mineralization level definition by means of the two-absorbing X-ray densitometer and marking out group of the greater risk of a foot mineralization disorder development at patients with diabetes.
Materials and methods
Decrease in a foot mineralization at diabetes patients conformed a mineralization of metatarsal bones below 0.50 g/cm^2 level and (or) tarsal bones below 0.69 g/cm^2 level.
Results
For risk assessment of development of foot mineralization disorder of patients were divided into groups, depending on age of demonstration group DM1 was made up of patients with DM demonstrations age below 20 years; the 2nd group – demonstration of 20–30 years; the 3rd group – demonstration above 40 years. At age of demonstration of DM till 20 years the smallest indicators of foot bone mineral density (BMD) in comparison with patients of 2nd and 3rd groups are revealed.
The disease experience did not differ significantly from that of 2nd and 3rd group patients. The relative risk in 1st group exceeded 1.0 but was not significant. At further risk analysis of violation of foot mineralization (in group of patients with age demonstrations for <20 years) the significant risk among the patients who have fallen ill aged before 14 years (OP $= 1.24$ ($1.28 \div 9.32$)) is revealed.
Output
The significant risk among the patients who have fallen ill aged before 14 years.

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Postprandial hypotension is attenuable with Acarbose treatment in older adults with diabetes mellitus type 2: a randomized controlled crossover cohort study

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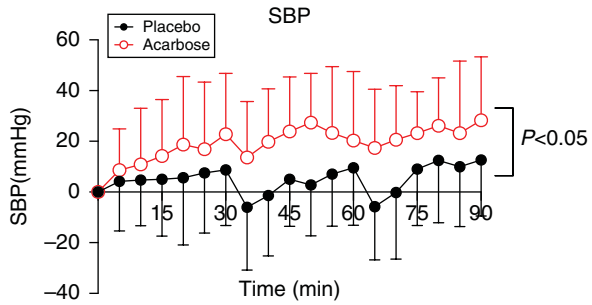
Postprandial hypotension (PPH) is common for older adults and may lead to syncope and falls. Adults with autonomic dysfunction, commonly those with diabetes mellitus (DM), are also at risk for PPH. Reported prevalence rates are 40% for PPH in DM patients. To date there are no reliable treatments for this condition in DM patients. It was our objective to demonstrate Acarbose, an α-glucosidase inhibitor, decreases the degree of PPH in an elderly DM cohort.
Fifteen adults (nine women and six men) with average age of 75.9 years (range: 67–85.2), BMI of 28.6 kg/m^2 (range: 20.1–35.5), and individual history of DM type 2 (duration: 9.0 years; hemoglobin A1C 6.8%) attended a treatment and placebo session (separate days at least 2 weeks apart) in random double-blinded order. Subjects were fed a standardized meal (4 °C) and then continuously monitored over 90 min for blood pressure by finometry, heart rate by electrocardiogram, and middle cerebral artery blood flow velocity by transcranial Doppler ultrasound (20-min baseline recorded for all measurements). Intravenous cannula was placed for blood glucose and catecholamine measurements.
Prevalence of PPH was 86.7% in DM subjects ($n=13/15$). The frequency of PPH occurring per study was 1.22 (range: 1–3) for Acarbose and 1.75 (range: 1–3) for placebo, and this difference was significant (t-test, $P=0.0359$). The hemodynamic response of systolic blood pressure (SBP) and mean arterial pressure (MAP) (baseline as covariant) was significantly different for subjects given Acarbose by mixed-model repeated measures two-factor (time and treatment) analysis of variance (SBP: $P=0.0248$, see figure; MAP: $P=0.0499$).
The reported higher prevalence of PPH in our study warrants further investigation. This is the first study to demonstrate Acarbose attenuates PPH in adults with DM. Our results suggest that Acarbose is a potential therapy for PPH in older adults with DM type 2.



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Effect of bariatric surgery on serum glucagon like peptide-1 concentration and metabolic parameters among obese type 2 diabetics

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Objective
Assess the effect of bariatric surgery on serum glucagon like peptide-1 (GLP-1) concentration and metabolic and biochemical parameters pre and postoperatively.
Methods
This prospective study comprised 50 subjects divided into 3 groups. Group 1: 20 obese subjects with T2DM (BMI >35) that underwent Roux-en-y gastric bypass surgery; Group 2: 20 lean subjects with T2DM and Group 3: 10 lean subjects as healthy controls.
Body weight, BMI and waist/hip ratio were analyzed. Fasting and 2hPP plasma glucose, HbA1c, lipid profile, fasting serum insulin and glucagon like peptide-1 (GLP-1) were tested.
For Group 1, tests were done twice, preoperative and 3 months after surgery. Insulin resistance was quantified using HOMA-IR.
Results
Fasting GLP-1 levels were lower in group 1 subjects compared to the other 2 groups (4.33±1.10 vs 5.21±0.89, 6.14±0.42 ng/ml, $P<0.001$). 2hPP GLP-1 levels were lower in Group 1 compared to the other 2 groups respectively (4.94±1.17 vs 6.04±0.99, and 9.32±0.97 ng/ml, $P<0.001$); also the difference between groups 2 and 3 was statistically significant. Fasting and 2hPP GLP-1 levels showed significant negative correlation with all metabolic parameters except HDL-C, which showed highly significant positive correlation. In Group 1, mean preoperative BMI was 47.3±4.2 vs 42.10±3.05, 3 months postoperatively. FBS was 145.9±19.2 vs 98.25±16.03 mg/dl, 2hPP was 208.9±33.2 vs 137.85±14.90 mg/dl, HbA1c was 9.6 vs 7.1% and fasting insulin was 20.9 vs 7.25 μIU, There were increase in the 2 hPP GLP-1 levels (4.9±1.2 vs 9.78±1.9 ng/ml) and HDL (34.1 vs 48.05 mg/dl). The level of significance was $P<0.001$ for all variables.
Conclusion
Bariatric surgery resulted in a statistically significant reduction in BMI and all metabolic parameters. 2h PP GLP-1 is low in diabetic patients. Postoperative 2hPP GLP-1 levels increased, possibly responsible for the metabolic benefits.

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Ileal interposition with diverted sleeve gastrectomy for treatment of type 2 diabetes

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Objective
To prospectively evaluate the results of laparoscopic ileal interposition (II) with diverted sleeve gastrectomy (DSG) for control of type 2 diabetes mellitus (T2DM) and related metabolic abnormalities.
Methods
All patients underwent II +DSG. They had T2DM ≥5 years with poor glycemic control despite adequate dosage of oral hypoglycemic agents (OHAs) and/ or insulin. The primary outcome was remission of diabetes (HbA1c <6.5% without OHAs/insulin). Secondary outcomes were reduction in antidiabetic agent requirement and components of metabolic syndrome.
Results
We report the postoperative follow-up data of 13.1±5.3 months (range: 3–26 months). There were 32 patients (male: female=21:11) with mean age of 48.7±7.8 (range, 34–66 years), duration of diabetes of 13.1±5.8 years (range, 5–30 years), and preoperative body mass index of 29.1±6.9 kg/m^2 (range: 22.4–39.5 kg/m^2). They had poorly controlled diabetes with mean FBS: 236.52±88.4 mg/dl, PLBS: 305. 1±124.3 mg/dl and HbA1c: 9.8±1.8%. Sixteen patients (50%) had hypertension, while dyslipidemia and microalbuminuria was present in 12 patients (39%) each.
The mean operative time was 387.7±84.3 minutes and the mean postoperative hospital stay was 8.8±5.4 days. Intraoperative complications were noted in 4 patients (12.5%). Nausea and loss of appetite was observed in 3 patients (10%), which improved over a period of 2 weeks. At 3 months postoperative follow up, none of these patients had any complications with regards to the intraoperative and immediate postoperative events.
Twenty two patients (70.5%) had diabetes remission. Fifteen/sixteen (93%) patients had remission in hypertension. All participants had weight loss ranging between 15 and 25%. Postoperatively statistically significant decline was observed in the glycemic and lipid parameters, microalbuminuria at all intervals ($P<0.05$). Patients with duration of follow up more than 6 months demonstrated to have better improvement in terms of reduction in glycemic, lipid parameters and microalbuminuria. Three patients had vitamin B12 deficiency 1 year after surgery.
Discussion
The surgery addresses the foregut and hindgut mechanisms for DM control. The DSG component restricts calorie intake and induces ghrelin (orexin) loss. It also excludes the duodenal loop, thereby negating the effect of insulin resistance promoting Rubino's factor. II leads to earlier and rapid stimulation of interposed ileal segment by ingested food resulting in augmented GLP-1 secretion.
Conclusion
II+DSG seem to be promising procedures for control of type 2 DM and associated metabolic abnormalities.

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Clinical usefulness of a bolus calculator in patients with type 1 diabetes mellitus treated with continuous subcutaneous insulin infusion (CSII)

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Objective
One of the major problems related to the use of CSII, or intensive insulin regimens in general, is the calculation of accurate insulin boluses. Abolus calculator incorporated into the insulin pump, estimates the dose of insulin to be administered at the meal on the basis of the following parameters: current blood glucose, grams of carbohydrate in the meal, carbohydrate to-insulin ratio (CIR), insulin sensitivity factors (ISF), target blood glucose and quantity of insulin previously administered, hereby making calculation easier and more precise.
The aim of the present study was to assess the efficacy of a bolus calculator incorporated into the insulin pump on pre and postprandial glycaemic control of patients with type 1 diabetes on continuous s.c. insulin infusion, evaluating changes in insulin dose, number of boluses administered, acute complications, glycemic variability and HbA1c, and quality of life.
Material y methods
We enrolled 20 subjects (8 men/12 women) older than 18 years old (mean age: 36 ± 12 years) with type 1 diabetes patients treated for more than 12 months (time on insulin pump therapy: 50 ± 32 months) with continuous s.c. insulin infusion (Minimed 722, Medtronic). The patients received a infrared-linked glucometer (Contour Bayer) so that glycemic values were directly transmitted to the pump to be used by the bolus calculator, with possibility to download all the recorded data. We calculate basal CIR and ISF for all the patients, who received all the information for the accurate use of the bolus calculator.
The following data were evaluated baseline and after 3 months using the bolus calculator: HbA1c, daily insulin dose(Basal and bolus), number of bolus/day, acute complications. Glycemiclability was evaluated using MAGE and S.D. from SMBG information, and quality of life (DQOL). A treatment satisfaction questionnaire was also evaluated.
Results
After 3 months using the bolus calculator we observed significant changes ($P < 0.05$) in weight (71.8 ± 8 vs 72.9 kg)and methabolic control (HbA1C 7.7 ± 1.1 vs $7.5 \pm 1\%$), we did not find differences in SMBG downloaded data (time in normo, hypo and hyperglucemia), S.D., MAGE, insulin daily dose, basal/bolus distribution, number of boluses/day, and DQOL.
Patients were satisfied with the bolus calculator, they find this option easy to use and accurate.
Conclusions
In a group of patients treated with continuous s.c. insulin infusion the addition of the use of a bolus calculator achieved an improvement in glycemic control with decrease of HbA1c levels, without changes in other studied parameters, with a high level of satisfaction of the patients.

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Effects of human insulin and insulin aspart preparations on levels of IGFI, IGFBPs and IGFI bioactivity in patients with type 1 diabetes

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Introduction
Type 1 diabetes (T1D) is characterized by primary insulin insufficiency and secondary disruption of GH–IGF–IGFBP axis. S.c. insulin therapy is necessary to normalize this axis. This study aimed to investigate whether the distinct insulin profiles obtained with insulin aspart and human insulin preparations, respectively, affect IGF1 concentration and bioactivity and IGFBP levels differently.
Methods
In a randomized, four-period crossover study, 19 patients with T1D received identical doses (0.2 U/kg s.c.) of insulin aspart, BIAsp70, or BIAsp50 immediately before a standardized meal, or human insulin 30 min before meal. Serum total IGF1, bioactive IGF1 and IGFBP-1 to -3 were measured for 9 h postprandially.
Results
After equipotent doses, IGFBP-1 levels decreased significantly during the first 3 h with all insulin treatments, but the AUC of IGFBP-1 was significantly higher in the BIAsp50 group (mean (range) 351, 312–396 μg h/l) as compared to insulin aspart (262, 233–294 μg h/l) and human insulin (256, 228–288 μg h/l, $P = 0.001$). Human insulin showed lower AUC of IGFBP-1 than insulin aspart preparations during 0–3, 0–6, 6–9 and 0–9 h. During the first 6 h, the four insulin preparations resulted in similar profiles and AUCs of total IGF1, bioactive IGF1, IGFBP-2 and IGFBP-3. Whereas total IGF1 remained constant, bioactive IGF-I fell at the end of the study (6–9 h), concomitant with the increase in IGFBP-1.
Conclusions
Despite distinct pharmacokinetic properties, the insulin aspart preparations had similar effects on IGF1 concentration and bioactivity, IGFBP-2 and IGFBP-3 as compared to those of human insulin, but differed in respect to IGFBP-1. Further, bioactive IGF-I appeared to be more sensitive to insulin exposure than total IGF1.

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Vitamin B_{12} deficiency in type 2 diabetic patients on metformin

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Introduction
Metformin is the only biguanide that is currently in use as an oral hypoglycemic agent in type 2 diabetes mellitus (DM). There are reports about the vitamin B_{12} deficiency in metformin users. This study was aimed to evaluate the vitamin B_{12} deficiency in patients with type 2 DM using metformin or other hypoglycemic agents.
Methods
A descriptive-comparative study was performed on 400 patients with type 2 DM presenting to Endocrinology Clinic since Jan 2011 to Jan 2012. The enrolled patients were divided in two groups: i) those receiving metformin from 6 months ago ($n = 200$); and ii) those receiving hypoglycemic agents other than metformin ($n = 200$). Blood samples were taken from all patients after 12 h fasting and serum vitamin B_{12} and other variables were measured and compared between two groups.
Results
Patients receiving metformin had significantly lower Vitamin B_{12} levels (320.94 ± 141.34 vs 408.50 ± 175.07 pmol/l, $P < 0.001$) and higher prevalence of vitamin B_{12} deficiency (14.5 vs 2%, $P < 0.001$). There was negative correlation between vitamin B_{12} levels with weight ($r = -0.18$, $P < 0.001$) and BMI ($r = -0.11$, $P = 0.02$) and positive correlation between vitamin B_{12} and metformin administration ($r = 0.26$, $P < 0.001$).
Conclusion
Patients with type 2 DM under long-term metformin therapy had lower vitamin B_{12} levels than those under other hypoglycemic drugs. The relation between vitamin B_{12} deficiency and metformin therapy indicates the need for periodic measurement of serum vitamin B_{12} level in patients under long-term metformin therapy.

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Medical staff experience and acceptance of an ICU insulin infusion protocol in a tertiary hospital in the Philippines

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Introduction
The recommended strategy for glycemic control among critically ill is i.v. insulin adjusted via a standardized insulin protocol. Critical to its success is acceptance of the implementing staff. In our hospital, we adapted and modified the Yale insulin infusion protocol (IIP). Evaluation of medical staff experience has not yet been done.
Objectives
To evaluate medical staff experience and acceptance of the IIP through a survey and focused group discussion.
Methods
A survey followed by focused group discussions among the medical staff of the Medical and Central Intensive Care Units were done. Questionnaires were

distributed to the nurses and medical residents of ICUs. Focused group discussions were done after to clarify the information derived from the survey.
Results
One hundred nine medical staff (47 nurses and 62 medical residents) participated in the study. Majority (76.7%) of ICU nurses felt they had good knowledge of the IIP. Seventy-seven percent of nurses agree that the IIP is effective in controlling hyperglycemia and 57.4% felt that it prevented hypoglycemia. While 74.5% held that the protocol increases their workload due to frequent glucose checks and need for computations to adjust the drip, majority (64%) agree that it is easy to administer. Seventy percent of nurses are satisfied with the use of the protocol. Similarly, most (80.6%) medical residents in the ICUs believe that the IIP is effective. While most felt that the protocol is not easy to administer (68%), majority (64%) would still opt to use it for their patients. The staff believes that periodic training and provision of supplies are key factors in improving the protocol.
Conclusion
Experience and acceptance of the insulin infusion protocol is generally excellent for both nurses and physicians. Despite an increase in workload, most believe the protocol to be effective and would advocate its use for ICU patients.

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Efficacy and safety of 1 year treatment with Liraglutide in subjects with type 2 diabetes

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Introduction
Liraglutide, a GLP-1 analogue, is a new option for the treatment of type 2 diabetes (DM2). The purpose of this study was to evaluate the efficacy and safety of liraglutide in daily clinical practice in a heterogeneous population with DM2.
Subjects and methods
Four visits were scheduled in a 1 year study (baseline, 4, 8 and 12 months). All patients with a HbA1c not on target (>7%) during an oral hypoglycemic treatment, or patients intolerant to metformin were recruited. Exclusion criteria were: the presence of kidney diseases on dialysis, lack of compliance or refusal to injective therapy. Changes in glucose, HbA1c, body weight, BMI and lipid profile were primary outcomes. Adverse events and drop-out rate were evaluated.
Results
243 subjects (110 males and 133 females) were recruited, with an age of (mean ± S.D.) 59.6 ± 10.4 years and a disease duration of 8.3 ± 7.0 years. Fasting blood glucose (10.3 ± 3.1 vs 8.4 ± 2.6 mmol/l, $P<0.0001$) and HbA1c (8.6 ± 1.3 vs 7.4 ± 1.0%, $P<0.0001$) decreased at 4 months and maintained a plateau overtime. Body weight (92.8 ± 18.9 vs 89.5 ± 18.2 kg, $P<0.0001$) and BMI (33.8 ± 6.6 vs 32.3 ± 6.0 kg/m^2, $P<0.0001$) decreased at 4 months and then remained stable. Lipids slightly decreased over time. Total cholesterol (4.6 ± 0.9 vs 4.3 ± 0.9 mmol/l, $P<0.01$), LDL-cholesterol (2.6 ± 0.8 vs 2.4 ± 0.7 mmol/l, $P<0.03$) and triglycerides (1.9 ± 1.0 vs 1.7 ± 0.9 mmol/l, $P<0.03$) decreased respect to baseline, independently of glucose and HbA1c changes. Conversely, HDL cholesterol increased (1.16 ± 0.28 vs 1.17 ± 0.27 mmol/l, $P<0.01$). 35 patients left the study, 3 of them because of adverse effects.
Conclusion
Liraglutide is effective in controlling DM2 in daily clinical practice. Liraglutide could have pleiotropic actions in the control of DM2.

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Metabolic surgery assessment score (MSAS): a tool to predict diabetes remission after modified bariatric surgery

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Objective
Laparoscopic ileal interposition (II) with sleeve gastrectomy (SG)/diverted sleeve gastrectomy (DSG) are types of modified bariatric surgery for treatment of type 2 diabetes (T2DM). DSG is preferred over SG in patients with less favourable metabolic profile. Owing to variable remission response in our patients, retrospectively we devised a novel score metabolic surgery assessment score (MSAS). It helps to select the type of procedure and to predict the diabetes remission before surgery.
Methods
Forty-six patients underwent II + SG and 29 for II + DS II + DSG was performed on 29 patients. MSAS was calculated based on preoperative parameters. MSAS of the subjects with and without remission (maintaining HbA1c <6.5% without any medication) were compared.
Results
Patients subjected to II + SG had mean age of 48.3 ± 8.1 years, duration of T2DM 9.8 ± 7.6 years and body mass index (BMI) 32.1 ± 6.9 kg/m^2. All patients had poorly controlled diabetes with HbA1C 9.5 ± 2.2%. Mean MSAS in patients who underwent II + SG ($n=46$) was 9.2 ± 1.4. Twenty one (46%) had remission in diabetes. In the same group, patients with BMI ≥35 kg/m^2, MSAS was 8.9 ± 1.7 and remission rate was 85%. MSAS was significantly lower in patients with remission than patients without remission (8.1 ± 0.8 vs 10.2 ± 0.9, $P<0.0001$).
Patients subjected to II + DSG had mean age of 48.7 ± 7.8 years, duration of T2DM 13.1 ± 5.8 years and BMI 29.1 ± 6.7 kg/m^2. All patients had poorly controlled diabetes with HbA1C 9.8 ± 1.8%. Mean MSAS in patients who underwent II + DSG ($n=29$) was 10.4 ± 1.3 (significantly higher than II + SG group, $P=0.0004$). Twenty one (72%) had remission in diabetes. MSAS was significantly lower in patients with remission than patients without remission (9.7 ± 0.8 vs 12.0 ± 0.5, $P<0.0001$).
Patients with MSAS ≥10 in II + SG group and MSAS ≥12 in II + DSG group did not get remission. MSAS was not significantly different ($P=0.1468$) in patients without remission in II + SG (10.2 ± 0.9) vs patients with remission in II + DSG (9.7 ± 0.8). This indirectly suggests that DSG instead of SG would have helped them in achieving remission.
Discussion
The surgery addresses the foregut and hindgut mechanisms leading to remission in T2DM. The SG component restricts calorie intake and induces ghrelin (orexin) loss.II leads to rapid stimulation of interposed ileal segment by ingested food leads resulting in augmented GLP-1 secretion. DSG leads to better remission by exclusion of Rubino's factor and GIP from duodenum, abolition of hedonic effect of food, earlier stimulation of ileum leading to better incretin response.
Conclusion
Preoperative MSAS can be a useful tool to select the type of surgical procedure and to predict post operative diabetes remission.

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No benefit for mortality but advanced risk of hypoglycemia with intensive glucose control in critical care unit brain injured patients: a meta-analysis of RCTs

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Background
Acute stroke, traumatic brain injury, and subarachnoid hemorrhage are common diagnoses of patients in Critical Care Units, which often lead to increased morbidity and mortality. Patients with brain damage experience wide glucose fluctuation, with episodes of hyperglycemia. Hyperglycemia has traditionally been associated with poor clinical outcomes after brain injury.
Objectives
To evaluate the effects of intensive versus conventional glucose control in mortality as well as the incidence of glucose disturbances and the neurologic outcome in critically ill brain injured adults.
Search methods
We performed a meta-analysis after systematically searching PubMed, and Scopus databases to retrieve RCTs in English. We initially retrieved 3081 citations. After removing duplicates, animal, pediatric, and studies including non ICU patients, eleven studies remained and were analyzed with Review Manager ver 5.1. Odds ratios (OR) or Peto Odds ratios (POR) with 95% confidence intervals (CI) were calculated.
Results
In critically ill adult patients, both in-hospital mortality, OR 1.05 (M-H, Fixed, 95%, CI 0.60, 1.85) and overall mortality, OR 1.09 (M-H, Fixed,

95% CI 0.88, 1.34) do not show any statistically significant difference ($P=0.86$ and $P=0.44$ respectively) in the two different strategies. Furthermore, no statistically significant difference occurred in neurologic outcome, OR 0.90 (M-H, Fixed, 95% CI 0.65, 1.24) $P=0.51$. However, there was a statistically significant difference in hypoglycemic episodes, POR 6.71 (P-Fixed, 95% CI 5.14, 8.75) $P<0.00001$, implying that aggressive treatment for lowering blood glucose may have deleterious effects.
Conclusions
Critically ill brain injured adult patients have more hypoglycemic events with intensive glucose control without neurological or survival benefit. Hypoglycemic events are not risk free and a tighter glucose control is not justified with the available data.

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Monitoring and control of blood glucose levels in patients with inefficiently controlled insulin-treated diabetes mellitus through the utilization of an on-line telemonitoring system

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Introduction
Our goal was to monitor and control the blood glucose levels in inefficiently controlled insulin-treated patients with diabetes mellitus (DM). The target was to achieve an early detection of DM deregulation, to maintain accepted HbA1c levels through active intervention and to minimize the outpatient department visits.
Description of methods/design
Twenty-six insulin-treated type 1 and 2 DM patients were enrolled (mean age 61.42 ± 14.5 years, mean BMI 31.67 ± 7.28 kg/m^2). Inclusion criteria were: insufficient control of DM, living far from specialized medical facilities or recent discovery/hospitalization of insulin treated DM. Data transmission from the glucose-meters (FreeStyle Precision and Free Style Freedom Lite, Abbott, Oxon-UK) to the computers of our clinic was done via modem, while storage, documentation and communication with the patients with e-mails or mobile-phone text messages was achieved through a specialized integrated software (Telemedicor, Hertfordshire-UK). Monitoring period was 6 months with measuring of HbA1c levels at enrollment and at 3 and 6 months afterwards.
Results
Statistical analysis (ANOVA) revealed a significant reduction of HbA1c at 3 (9.92 ± 2.79 vs $6.68\pm0.92\%$ $P<0.0001$) as well as 6 months (9.92 ± 2.79 vs $6.61\pm0.88\%$, $P<0.001$) from the time of enrollment. The reduction of HbA1c was greater in the group of patients with an initial HbA1c level above 10% at 3 (12.11 ± 2.2 vs 6.65 ± 1.19 $P<0.0001$) as well at 6 months (12.11 ± 2.2 vs 6.55 ± 1.09 $P<0.0001$) compared to the group with an initial HbA1c level below 10% at 3 (7.74 ± 0.97 vs 6.70 ± 0.58 $P<0.001$) and 6 months (7.74 ± 0.97 vs 6.68 ± 0.64 $P<0.001$).
Conclusions
Telemonitoring DM patients can result in improved compliance due to a more frequent doctor-patient contact. This is reflected in the reduction of HbA1c levels. In addition, a reduction of visits of outpatients department is achieved resulting in lower cost and less patient inconvenience.

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Insulin detemir has similar effect when using single daily dose with same dose twice daily as basal insulin therapy with oral antidiabetics.

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Aim
To compare the effectiveness of insulin detemir when added to oral antidiabetic drug therapy as a single daily dose or twice daily.
Method
Fifty patients with a diagnosis of type 2 diabetes receiving oral antidiabetic drug therapy and glycemic control did not provided were included the study. Insulin detemir was begun 0.1–0.2 U/kg daily dose, half of the patients started with a single daily dose and the other half with same dose twice daily. At the end of the twelfth week, the groups were crossing. Insulin doses was titrated every 2 weeks for 24 weeks according to capillary blood glucose including in the morning and in the evening levels.
Results
Fifty patients completed the study, mean age was 55.5 years (M/F: 26/24), mean duration of diabetes was 7.0 years and initial HbA1c mean was $9.5\%\pm1.2$. Initial mean HbA1c was $9.8\%\pm1.3$ in the group receiving single dose (Group I), and was $1.5\pm9.3\%$ in the double-dose group (Group II). At the end of 12 weeks, improvements in HbA1c levels were observed 1.8% in Group I and 1.5% in Group II ($P=0.6$). Change in A1c levels in the next 12 weeks after cross-over, were -0.2% and -0.1% in Group 1 and Group 2 ($P=0.6$). While the initial daily insulin doses were 0.11/kg in both groups, at the end of the first twelve weeks it was 0.29 in the Group I, and 0.32 in the Group II ($P=0.2$). After crossover, daily insulin doses were 0.42 and 0.38 ($P=0.3$) respectively. Daily insulin dose was only about 10% higher in double-dose periods and was not statistically significant in pre and post crossover periods.
Conclusion
Reason for the difference in dose requirement may be due to comfortable and easily titration ability feature of double daily dose. According to the results obtained in this study, a single daily dose of insulin detemir has a similar effect at the same dose of twice daily when used as basal therapy with oral antidiabetics.

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P472

Factors related to nonadherence with diabetes treatment among Brazilian type 2 diabetes mellitus patients

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Introduction
The patients' nonadherence to treatment is a universal problem and has several related factors. Knowing these factors can provide information for planning of health actions. The aim of this study was to relate the prevalence of nonadherence to treatment for diabetes with sex, age, years of education, duration of diabetes and metabolic control.
Description of methods/design
This is cross-sectional study. The data were derived from the database of a cross-sectional study conducted in 2010 in 17 health units from Passos' city, Minas Gerais. The sample consisted of 417 patients who had no treatment adherence. For analysis, we used descriptive statistics.
Results
Regarding drug treatment, the prevalence of nonadherence was higher among men (17.0%) in patients with 60 years old or older (17.1%), 4 to 8 years of education (18.3%), <10 years of diagnosis (16.0%) with inappropriate glycated hemoglobin (HbA1c) (18.2%), triglycerides (18.0%), HDL cholesterol levels (17.2%), normal total cholesterol (18.4%) and LDL levels (17.9%). Regarding diet, the prevalence of nonadherence was higher among women (98.6%) in patients with 60 years old or older (98.5%), <4 years of education (98.9%), <10 years of diagnosis (98.4%) with inappropriate total cholesterol (99.0%), HDL (98.4%) and LDL cholesterol levels (98.4%), normal HbA1c (99.0%) and triglycerides levels (98.7%). Regarding physical activity, the prevalence of nonadherence was higher among women (43.5%), in patients under 60 years old (44.0%), 4 to 8 years of education (42.3%), ten or more years of diagnosis (42.2%) with inappropriate HbA1c (45.6%), total cholesterol (45.6%), HDL cholesterol levels (43.4%) and normal LDL cholesterol levels (46.4%).
Conclusion
The nonadherence to treatment was higher among women, patients aged 60 years or older, 4 to 8 years of study, <10 years of diabetes, HbA1c, total cholesterol and HDL-C above recommended, appropriate values of LDL-C.

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P473

Cardiovascular effects of treatment with Liraglutide in a population with type 2 diabetes

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Introduction
Liraglutide, a human GLP-1 analogue, is a new option for the treatment of type 2 diabetes (DM2). The purpose of this study was to evaluate the effects of liraglutide on cardiovascular risk factors in daily clinical practice in a heterogeneous population with DM2.
Subjects and methods
Four visits were scheduled in a 1 year study (baseline, 4, 8 and 12 months). Patients with a HbA1c not on target (>7%) during an oral hypoglycemic treatment, or patients intolerant to metformin were recruited. Exclusion criteria were: the presence of kidney diseases on dialysis, lack of compliance or refusal to injective therapy. Changes in systolic (SBP) and diastolic blood pressure (DBP) and cardiovascular risk scores (Framingham and UKPDS algorithms) were evaluated. 243 subjects (110 males; 133 females) were consecutively recruited (age, mean ± S.D. 59.6 ± 10.4 years; disease duration 8.3 ± 7.0 years).
Results
Both SBP (154.7 ± 22.0 vs 143.5 ± 23.6 mmHg, $P<0.0001$) and DBP (89.1 ± 11.5 vs 84.4 ± 10.7 mmHg, $P<0.0001$) decreased respect to baseline. Both reductions were independent of changes in glucose, HbA1c and antihypertensive drugs. Framingham risk score decreased at 12 months respect to baseline (30.2 ± 17.9 vs 35.6 ± 20.3%, $P<0.0001$). At the same time a reduction in the risk of coronary events (15.7 ± 11.4 vs 20.7 ± 15.2%, $P<0.0001$) and of fatal coronary events (10.9 ± 9.5 vs 15.2 ± 13.8%, $P<0.0001$) was recorded. Finally, also the risk of stroke (8.6 ± 8.5 vs 12.0 ± 15.3%, $P<0.0001$) and fatal stroke (1.4 ± 1.5 vs 2.3 ± 3.3%, $P<0.0001$) at 12 months decreased respect to baseline. During the 12 months of observation no cardiovascular accidents occurred.
Conclusion
Liraglutide is effective in reducing blood pressure and cardiovascular risk in patients with DM2 in a relatively short-time treatment.

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P474

Therapeutic swicht at discharge improves medium term metabolic control in patients with type 2 diabetes and high vascular risk

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Objective
To evaluate the characteristics and glycemic control in admitted patients with type 2 diabetes and high vascular risk. To analyze therapeutic switch effect at hospital discharge on medium term metabolic control.
Methods
Cross-sectional study including patients with type 2 diabetes and high vascular risk. Demographic and clinical characteristics, laboratory parameters, and diabetes treatment at baseline and discharge were analized. We used a specific insulin protocol during admission, switched treatment at discharge in selected cases and evaluated the metabolic control evolution 3 months after discharge.
Results
126 patients were included (55.6% male, mean age 64.3 years and mean duration of diabetes 16.3 years). 63.5% had hypertension, 47.6% dyslipidemia and 19% were smokers. 7.1% had a history of stroke, 40.5% coronary heart disease and 36.5% peripheral arteriopathy. Most prevalent causes of admissions: 22.2% amputation, 17.5% coronary bypass, 15.9% valve surgery and 15.1% coronary heart disease. Prior to admission, 38.9% were treated with insulin, 30.2% with oral hypoglycemic agents (OHA) and 25.4% with combinated therapy. At discharge, 45.2% remained their previous treatment, adjusted if necessary and 43.5% started insulin treatment (8.7% basal insulin plus OHA, 7.1% basal insulin, 11.9% biphasic insulins, and 15.9% basal-bolus regimen). Mean HbA1c at admission decreased from 8.9% (18.6% patients, <7%) to 7.49%, (44.2% patients <7%) ($P<0.001$) 3 months after discharge, with no significant differences detected in the rest of variables analized.
Conclusions
Most hospitalized patients with type 2 diabetes presented with poor glycemic control and chronic complications. Insulin consensus protocol can improve glycemic control during hospitalization. Optimizing therapy at discharge improves medium term metabolic control.

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P475

Abstract withdrawn.

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P476

Impact on weight and glycemic control in patients with type 2 diabetes and obesity treated with liraglutide

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Background and objectives
Evaluate the impact of therapy with GLP-1 analogues (liraglutide) on glycemic control and weight in patients with type 2 diabetes and poor metabolic control.
Material and methods
We conducted a retrospective observational study analyzing 67 patients with type 2 diabetes mellitus and BMI >30 kg/m^2, over 6 months of treatment. We evaluated HbA1c and weight at 3 and 6 months, performing statistical analysis with SPSSv18 using the Student's *t*-test for paired data.
Results
The mean age was 50.29 ± 13.2 years, being women the 50.7% of them. The average weight at baseline was 17.03 ± 104.26 kg, BMI 38.5 ± 5.99 kg/m^2 and mean HbA1c 8.4 ± 1.3%. Initially 35.8% used oral antidiabetic monotherapy, 34.3% dual therapy with OAD, 9% triple therapy and the 20.9% remaining were treated with OAD plus insulin. Mean HbA1c at 3 months was 6.7 ± 1.09%, and at 6 months was 6.5 ± 1.2%, evidencing a statistically significant and clinically relevant decrease of mean HbA1c at 3 months of 1.81% ($P<0.000$) and at 6 months of 1.3% ($P<0.000$). The average weight at 3 months was 99.2 ± 15.48 kg and at 6 months of 94.18 ± 15.48 kg. We observed a weight loss of 7.46 ± 5.8 kg ($P<0.000$) after 3 months. At 6 months, there was a weight reduction from baseline of 13.5 ± 16.5 kg ($P<0.000$). There were mild gastrointestinal secondary effects in six patients that entailed the abandonment of treatment in three cases.
Conclusions
A clinically relevant reduction of HbA1c and weight were observed. The association of liraglutide to conventional diabetes treatment in type 2 diabetic patients with obesity confers a benefit on glycemic control and weight loss.

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P477

Short-term, temporary insulin degludec, an ultra-long-acting basal insulin, as compared with glargine on the glycemic control in Japanese patients with type 2 diabetes

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Introduction
Insulin degludec (IDeg) is a new-generation insulin that forms soluble multi-hexamers after s.c. injection, resulting in ultra-long duration of action.

Objective
We investigated the efficacy of glycemic control and safety on Japanese patients with type 2 diabetes who treated temporarily with IDeg or glargine and achieved target glucose level.
Research design and methods
Ten Japanese subjects with type 2 diabetes (male/female; 8/2, Age; 59.8±12.1 years, disease duration; 3.9±4.2 years, HbA1c; 9.0±2.4%, BMI; 26.7±3.7, FPG; 179±49 mg/dl, 2hPPG; 291±123 mg/dl, FCPR; 2.3±1.3 ng/ml) were treated with a short-term, 3–4 week course of IDeg to achieve a target 2hPPG of <140 mg/dl without oral medication in admission. They were initially treated with 4 unit of IDeg and titrated according to the treat-to-target protocol. Efficacy and safety of short-term IDeg therapy were retrospectively compared with conventional basal insulin glargine therapy. Glycemic control after withdrawal of IDeg or glargine was also followed up.
Results
FPG of IDeg-treated group decreased to <100 mg/dl in 8.8±2.2 days and remained in a flat and stable range. Maximum dose of IDeg to achieve target blood glucose level was 23.0±4.8 unit/day on average. Blood glucose levels of IDeg-withdrawn patients were kept without medication or with only oral antidiabetic drugs. Hypoglycemic episodes were observed 3 times in a patient treated with IDeg, which were due to inappropriate exercise before meal. Treat-to-target protocol of IDeg provided prompt and fine glycemic control. Body weight was decreased by 2 kg on average with temporary IDeg therapy.
Conclusion
Short-term, temporary basal insulin IDeg therapy improved glycemic control in Japanese patients with T2DM.

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P478

Perioperative liraglutide therapy for orthopedic patients with T2DM

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Introduction
Diabetic patients with limited exercise during orthopedic perioperative period tend to gain weight, and their glycemic control frequently deteriorates. However, temporary insulin therapy has a risk of hypoglycemia. To investigate the efficacy and safety of perioperative liraglutide therapy, we performed a retrospective case analysis.
Research design and methods
Twenty one Japanese patients with T2DM (male/female; 9/12, Age; 68.2±14.7 years, HbA1c; 8.0±1.2%, BMI; 26.3±2.7) were initially treated with 0.3 mg of liraglutide and allowed to dose-titrate up to 0.9 mg/day before elective orthopedic operations, e.g. spine surgeries (9 cases), artificial knee joint replacement (9), bone fracture surgeries (2), and amputation surgery of toe necrosis (1). In case of hyperglycemia (blood glucose level is over 200 mg/dl) during operation, regular insulin was added. Change in body weight, fluctuation of glycemic level, and perioperative complications were analysed.
Results
After initiation of liragludite therapy, the body weight decreased in 3.3±2.0 kg before operation. Liraglutide therapy achieved good glycemic control throughout the perioperative period. One patient suffering painful knee osteoarthritis lost weight with preoperative liraglutide therapy, and eventually he avoided the knee operation because his knee pain disappeared. Two patients could not increase dose-titrate up to 0.9 mg because of nausea as a side effect. Additional regular insulin was not needed except for 5 patients who were prescribed only 4 unit of insulin during operation. Twenty out of 21 patients withdrew liraglutide therapy after operation and rehabilitation. Hypoglycemic episodes, retardation of wound healing, or other complications were not observed.
Conclusion
Liraglutide provides an effective and optional way to safely achieve good glycemic control in perioperative for orthopedic operations subjects with T2DM, especially those with limited exercise ability and those at risk of hypoglycemia.

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P479

Insulinator: a computer program to help in the insulin therapy

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Introduction and objectives
In clinical practice many professionals are involved in the diabetes treatment. To facilitate this work, we present a computer program, based in recommended guidelines, to improve the management of insulin therapy, called insulinator. We evaluated the correlation between these and clinical practice.
Materials and methods
Insulinator has been made so that using a number of variables (weight, HbA1c and glycemic control) offer an orientation on the insulin therapy dose. This program present several regimens (basal insulin, mixtures, and basal-bolus regimen). You can start insulin, adjust the same regimen and increase to superior regimen. From 250 clinical histories, we compared the regimen and total dose introduced by endocrinologists in clinical practice, with virtually provide this software tool. The main variable is the concordance between both, considered as such an approximation of ±15% of the total dose of insulin.
Results
Adjustment of the same regimen: C (concordance) L (lower dose) H (higher dose).
1 Basal insulin (29): C 93.10%, L 3.45%, H 3.45%.
2 Basal-bolus therapy (43): C 90.70%, L 4.65%, H 4.65%.
Intensification to a superior regimen.
C (concordance) L (lower dose) H (higher dose).
1 Start basal insulin (36): C 52.78%, L 33.33%,H 13.18%.
2 Start Basal-Bolus regimen (60): C 33.33%, L 48.33%, H 18.33%.
3 Basal insulin to basal-bolus regimen (20): C 80.00%, L 5.00%, H 15.00%.
4 Mixtures to basal-Bolus regimen (62): C 79.03%, L 9.84%, H 11.29%.
Conclusions
Insulinator is a useful tool in insulin manegement, showing concordance with useful clinical practice. We appreciate that the adjustment presents a very high concordance. However in the intensification this concordance is less, because it tends to underestimate the final dose of insulin. This has been intentionally in this direction to reach the same point but more prudently. Finally will be the professional who, based on calculations recommended by the program and considering the individual patient factors choose the final dose and insulin regimen.

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P480

The long term effect of metformin plus DPP-4 inhibitor switching from metformin plus pioglitazone combination therapy in type 2 diabetes

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Two years ago, we presented the short term 6 month results of metformin plus DPP-4 inhibitor combination therapy when we can't reach the target below 7% of HgA1c with metformin plus pioglitazone combination that is best in terms of relieving insulin resistance in early diabetes.
Switching the pioglitazone to the DPP-4 inhibitor that improves insulin secretory dysfunction can be the next useful step to attain glucose control goal and DPP-4 inhibitors that increase insulin secretion by glucose dependent manner can also relieve insulin resistance because they improve first phase insulin secretion defect and prevent late hyperinsulinemia. DPP-4 inhibitor is also better than pioglitazone in weight aspect.
Total 111 patients were followed by 26.5 month ($\pm$9.7) after switching from metformin plus pioglitazone.
Sulfonylurea or pioglitazone was added for adequate glucose control in 14% (15/111) and the dose of DPP-4 inhibitor was decreased in 6% (7/111). The mean increasing dose of metformin to maintain HgA1c target who maintained metformin plus first dose of DPP-4 inhibitor in 80% (89/111) was 260 mg in the end.
HgA1c was improved in 76% (68 of 89 who maintained metformin plus first dose of DPP-4 inhibitor) from 7.40 to 6.66% and the metformin dose was increased by 240 mg. HgA1c was aggravated in 22% (20/89) from 6.85 to 7.22%.
HOMA-IR was improved in 46% (0.91$\pm$0.92) and aggravated in 53% ($-$1.25$\pm$1.17). Mean HOMA-IR change was $-$0.24$\pm$1.51.
Mena weight reduction was 2.23 kg ($\pm$3.17). The weight was decreased in 72% (3.63$\pm$2.51) and increased in 19% (1.77$\pm$1.73).
Although dose of metformin was slightly increased to maintain glucose control target with time, metformin plus DPP-4 inhibitor can be a good treatment option in maintaining insulin resistance as well as glucose and weight control as compared to Metformin plus pioglitazone therapy.

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P481

Protective effects of *Swietenia macrophylla* King (seed & endocarp) aqueous-methanolic extract on pancreatic islet histology in streptozotocin-induced diabetic rats

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The oral antidiabetic drug, glibenclamide, stimulates the insulin producing β-cells constantly through a harsh mechanism which eventually may permanently reverse its endocrinofunction. The study investigates the protective effects from Swietenia seed and endocarp aqueous-methanol extract on pancreatic islets histology in rats of type 2 DM model. Phytochemical analyses were performed prior to the *in vivo* study to screen the aqueous-methanolic extract of the combined plant parts. The experimental groups were rendered diabetic by chemical combination of STZ (65 mg/kg bwt, i.v.) and NAD (230 mg/kg bwt, i.p.) in adult rats. Diabetic rats were orally force-fed with glibenclamide (5 mg/kg bwt) or extracts (250 mg/kg bwt) daily for 3 weeks. Body weight (g) and FBG levels (mg/dl) were determined at treatment intervals of 0, 7, 14 and 21. Subsequently, Langerhans' islets were examined by H&E staining. Photomicrographs of pancreatic islet revealed that administration of extracts showed improved cellular density suggesting that the extracts were capable of inducing β-cells recovery and/or regeneration following destructive effects of STZ. Findings indicate that *Swietenia macrophylla* King seed and endocarp aqueous-methanolic extract exhibits protective effect on islet histology and were also involved in correction of altered biological parameters. Hence, it may serve as candidate for developing a safe, complaisance and promising neutraceutical for the management of diabetes.

Key Words
Swietenia macrophylla King, Diabetes, islet of Langerhans, Streptozotocin, Rats.

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P482

Are there gender differences in liraglutide response in adults with type 2 diabetes?

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Background
Recent reports suggest that female sex could be an indicator of better response to liraglutide in patients included in clinical trials.
Aim
To compare efficacy of liraglutide in women and men with type 2 diabetes mellitus (T2DM), in real clinical practice.
Patients and methods
Our study included 116 patients with T2DM and obesity (BMI $>$30) attending outpatient clinic, which initiated with liraglutide (0.6 mg/daily for the first week, followed by a dose of 1.2 mg/daily) and were studied prospectively during 6 months. Demographic, clinical and biochemical characteristics were collected at baseline and after 6 months. The percentage of patients achieving HbA1c $<$7% with no weight gain was assessed (responders). Data are expressed as mean (S.D.) or as percentage.
Results
There were 49.1% men. At baseline the 2 groups were similar in age, years of evolution of DM, glycemic control and weight. At 6 months women and men showed significant reductions in weight and HbA1c from baseline (women: IMC 41.9 (8.6) vs 39.6 (7.2), $P<0.001$. HbA1c 7.8 (1.4) vs 6.8 (1.1), $P<0.001$. Men: IMC 37.4 (6.3) vs 36.0 (5.9), $P<0.001$. HbA1c 8.0 (1.3) vs 6.8 (1.1), $P<0.001$). There were no differences in the improvement of glycemic control between sex groups. The reductions in body weight were slightly greater in women but the difference was not statistically significant with men. There were more 'responders' between women but not statistically different (47.1 vs 62.9%, *P*: 0.187). Significant reductions in lipid and blood pressure occurred from baseline, but differences between the 2 groups were non-significant. No major hypoglycemia and a low incidence of minor hypoglycemia were seen in both groups.
Conclusions
This study demonstrates that liraglutide, in a real world setting, is associated with reduction in HbA1c, body weight, blood pressure and lipids in both sex groups. We did not find differences in liraglutide response between women and men.

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Degree of glycemic control in treated type 2 diabetic patients who live in a district of Madrid, Spain

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There is a direct relation between the degree of glycemic control and the incidence and progression of diabetic complications. In addition improving glycemic control improves diabetic complications.
Aim
To assess the degree of glycemic control, according to published guidelines, in treated type 2 diabetes mellitus patients who live in a district of Madrid, Spain.
Methods
Cross-sectional study in 501 consecutive patients with T2DM who attended regularly our outpatient clinic of Endocrinology in Madrid, Spain for a routine follow-up visits. In addition to routine ingredients glycated haemoglobin (HbA1c) and fasting plasma glucose (FPG) were measured in all of the subjects after and overnight fast. Only 353 of 501 patients were self-monitoring capillary glucose by home reflectance meters. The postprandial capillary glucose (PCG) was the mean of the last postbreakfast, postlunch and postdinner capillary determinations. On the basis of recommendations for adults with T2DM from the ADA, we applied the following goals for our comparisons: HbA1c $<$7.0%, FPG $<$7.2 mmol/l and PCG $<$10.0 mmol/l).
Results
Mean ($\pm$S.D.) age was 65.4 $\pm$ 11.9 years, 218 (44%) were male. Ninety-six (19%) met coronary artery disease (CAD). Overall, 56% patients received insulin therapy alone or both insulin and oral hypoglycaemic drugs (OHD), and the remaining 44% took taking OHD alone. Only 41% of patients meet the recommended ADA target of HbA1c $<$7%, the percentage was higher among subjects taking OHD than in those on insulin (48 vs 30%; $P=0.007$), whereas no

significant difference for HbA1c was found between individuals with and without CAD. More patients reached the target for PCG than for FPG (65 vs 27%) and there were no differences between the treatment subgroups.
Conclusion
The poor glycemic control observed in the diabetic population studied, supports the need for more aggressive treatment in these patients to achieve the goals recommended by the accepted guidelines.

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P484

Copeptin: may it be a novel biomarker for insulin theraphy in subjects with diabetes?

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Background and aim
As a prognostic marker, copeptin levels were independent predictors of survival in critically ill patients suffering from hemorrhagic and septic shock. Copeptin levels also have prognostic implications in chronic diseases other than acute illness. The stress-mediated activation of the HPA axis may be have role in the pathogenesis of the insulin resistance, metabolic syndrome, and diabetes. Furthermore, AVP action has been linked to liver glycogenolysis and insulin and glucagon secretion. We tested the hypothesis that plasma copeptin would be associated with stress, diabetes, and treatment of diabetes.
Materials and methods
Healthy male Wistar rats, about 3 months old, weighing 200–250 g, were obtained from University Animal House. They were housed in small cages at standard conditions (24±2 °C and 50±5% humidity) with a 12 h light:12 h darkness cycle and were fed *ad libitum* with standard rat chow and tap water. Rats were divided into four groups: eight control (C), eight diabetic (D), eight diabetic+ insulin (DI) and eight stress (S) rats. Blood samples were collected into plain tubes (1.5 ml) and serum was separated via centrifugation at 1500 r.p.m. for 15 min. Samples were stored at −86 °C until analysis. Quantitative measurement of copeptin was performed using the ELISA method (Uscn Life Sciences, USA), according to the manufacturer's instructions.
All values are expressed as the mean±s.d.. The data were evaluated by one-way ANOVA and *post hoc* least significant difference (LSD) for multiple comparisons of the means. P values <0.05 were considered significant.
Results
There was not significant difference in terms of serum copeptin levels among groups except diabetes+insulin group.
Conclusions
Copeptin may be considered as a new tool for the comparison of the efficiencies of new therapeutic modalities in diabetes.

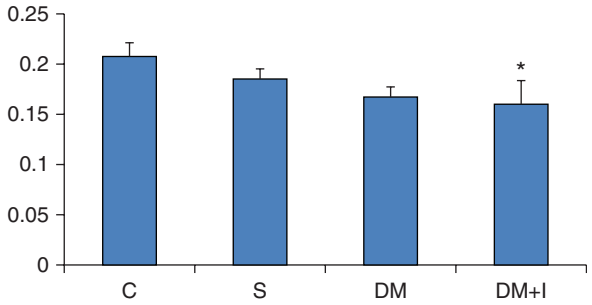

Figure 1 ELISA results of copeptin. C, control; S, stress; DM, diabetes mellitus; DM+I, diabetes mellitus+ insulin; *$P<0.05$.

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P485

Efficacy and safety of liraglutide in morbid obese patients in first year of commercialization in Spain

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Aim
To evaluate the efficacy and safety for first patients treated in our clinic with liraglutide.
Methods
Retrospective study of patients who started treatment with liraglutide in the last year in our department. We collected data on age, years of evolution, chronic complications, previous treatment, physical examination, analytical parameters and side effects.
Results
Fifty-four patients. Follow-up: 4–12 months. Mean age: 53.8 (s.d. 11.9) years, 44.8% men. Pretreatment: 37% insulin and OADs, 48.1% only OADs and 14.8% without treatment. There were significant differences between initial and final weight 116.5 (s.d. 34.12) vs 110.94 (s.d. 27.9) kg (P 0.007), mean blood glucose 150.81 (s.d. 56.49) vs 117.0 (s.d. 49.02) mg/dl (P 0.006), HbA1c: 7.24 (s.d. 1.67) vs 6.35 (s.d. 1.22)% (P 0.00), uric acid 5.93 (s.d. 1.66) vs 5.24 (s.d. 1.44) mg/dl (P 0.04), C Peptide: 2.88 (s.d. 1.10) vs 5.52 (s.d. 3.69) (P 0.04) and waist circumference 131.57 (s.d. 30.44) vs 124.14 (s.d. 21.48) cm (P 0.05). There were no differences in systolic or diastolic BP, LDL-c, HDL-c, TG, TG/HDL-C, creatinine, microalbuminuria, liver enzymes, TSH or calcitonin. The average weight loss was 5.62 (s.d. 6.86) kg, significantly higher in men than in women (8.93±6.96 vs 0.65±2.27, P 0.007), and correlated negatively with age (0.05) and positively with weight at the beginning (0.00). The mean decrease in HbA1c was 0.88±0.77 and correlated positively with baseline HbA1c (0.001). There was no correlation between the decrease in HbA1c and weight loss (0.31). Side effects: 4 patients reported nausea or postprandial discomfort that in 1 case led to suspension. No hypoglycemia or other side effects occurred.
Conclusion
i) Liraglutide treatment in obese patients with T2DM achieve an effective reduction of weight, waist circumference and HbA1c, with scarce sides effects.
ii) Patients who might benefit more from this treatment would be men with higher HbA1c and weight.

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P486

Evaluation of adolescents with type 1 diabetes after transition from paediatric to adult care

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Objective
Our objective in this study was to identify the type of clinical care received by young type 1 diabetic patients who have made the transition from paediatric to adult care, and to assess the metabolic status of the patients. The research aimed to develop a sustainable and coordinated approach to facilitating the transition between diabetes services for adolescents and reveal from the perspective of the adolescents living with type 1 diabetes their experiences surrounding the process of transition.
Material and methods
We evaluate all the patients transferred to our adult unit during the last year. Twenty-three type 1 diabetic patients were analysed. A questionnaire was used to evaluate the opinion of the patients concerning the transitional process.
Results
Twenty-three type 1 diabetic patients (63% F/36% M) with mean evolution of diabetes 9.5 years (6–14 years). Mean BMI was 23.68 kg/m^2, having 44.4% of the patients BMI higher than 25 kg/m^2. Mean HbA1c was 7.58%(6.5–8.6%).The patients were treated with MDI (18.18% with NPH and rapid insulin and 81.8% with glargina/Detemir and rapid insulin). Nine percent had incipient nephropathy. No other chronic complications were found. The patients were quite satisfied with the transitional process.
Conclusions
Transition marks a critical phase for young, diabetic patients as they may frequently switch from one physician or centre to another. The individual optimization of therapy, established during paediatric care, provides the decisive

groundwork for disease control in young adults. It's importantant to prepare, coordinate and evaluate transitional processes between paediatric and adult units.

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P487

Insulin pump therapy as an obligatory treatment modality in a type 2 diabetic patient

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Introduction
Continuous s.c. insulin infusion (CSII) therapy is a well documented treatment modality in type 1 diabetes mellitus (DM), but its usage in type 2 DM has not become prevalent so far. It may be a treatment alternative in severely insulin resistant type 2 diabetics recieving high doses of insulin. We present a type 2 diabetic patient that we had to shift from multiple dosage insulin (MDI) injection to CSII therapy due to the widespread echimosis at the insulin injection sites.
Case
Fifty-seven-year-old female patient with type 2 diabetes for 19 years was on insulin therapy for 6 years of the last four being as MDI therapy. She describe decchymosis at insulin injection sites from the onset of insulin therapy. Her hematological tests for coagulopathy were completely normal. Due to the injection site problems her glycemic control could not be achieved (frequent hypoglycemic and hyperglycemic episodes) with MDI after which switch to CSII therapy was done. Her compliance to CSII was well and she didn't experience large glucose fluctuations during with this therapy anymore. After initiation of CSII, her daily insulin need decreased and HbA1c improved markedly (Table).

Table 1 Table: HbA1c and total dialy insulin need before and after CSII

	Pre-CSII	Post-CSII (3rd month)	Post-CSII (9th month)
HbA1c (%)	7.2	6.4	6.1
Meandailyinsulin (units/day)	54	32.4	32.4

Conclusion
Studies about CSII therapy in type 2 DM have demonstrated that this therapy improves the gycemic control, decreases the insulin need and HbA1c of the patients. In addition less blood glucose fluctuations and better quality of life measures were recorded with this treatment modality. Insulin administration with CSII seems to be an alternative in selected type 2 diabetics as in our case.

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P488

Absence od metformin therapy and inadequate insulin doses are the basic features of diabetic patients with unregulated diabetes

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Introduction and objectives
The aim of our study was to determine which characteristics of the uncontrolled diabetes are among randomly selected patients with type 1 (T1DM) and type 2 (T2DM) diabetes.
Material and methods
A total of 194 diabetic patients were observed, 142 with T2DM and 52 with T1DM, recruted in outpatient clinic. Patients were divided into three groups according to HbA1c values: $<7\%$ (G1), 7–8% (G2) and $>8\%$ (G3). Statistical analysis: *t*-test, test of proportions, multiple logistic regression analysis.
Results
Patients (G1–G3) did not differ by age, prevalence of types 1 and 2 diabetes: T1DM (59.6%; 25%; 15.4%) and T2DM (55.3%; 23.3%; 21.3%) and disease duration. G1 compared to the G3 significantly differ or with the tendency of significance in representation of males (38.6 vs 56.3%, *P* 0.02), triglyceridemia level (1.8 ± 1.4 vs 1.4 ± 0.9 mmol/l, *P* 0.10), fibrinogen (3.7 ± 0.8 vs 3.4 ± 0.8 g/l, *P* 0.05) and platelets (259.3 ± 60.5 vs $230.1\pm60.7*10^9$/l, *P* 0.02) were lower (165.5 ± 10.3 vs 169.5 ± 10.9 cm, *P* 0.06), with less the used biguanides (45.6 vs 65.6%, *P* 0.07) and especially sulphonylurea (23.7 vs 46.9%, 0.02), and more were on insulin therapy (84.2 vs 53.1%, $P<0.01$), with multiple daily insulin dose (49 ± 2 vs 37 ± 12, *P* 0.01) and more frequent retinopathy (57.1 vs 36.7%, *P* 0.07). G1 to G2 patients were more often on insulin therapy (81.3 vs 53.1%, *P* 0.01).
Conclusions
Patients with glucose unregulation were characterized with clusters of components that are the part of increased insulin resistance (increased insulin dose…) and with simultaneous therapeutic inconsistency to it (metformin therapy). As a consequence, there were increased level of hemostatic components (platelets, fibronogen) and increased incidence of diabetic retinopathy.

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P489

Analysis of the newly diagnosed diabetic patients, one year and five years of evolution, C-peptide, autoinmunity, treatment and metabolic control

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Objective
We retrospectively analyzed patients with type 1 diabetes onset, attended during the years 2005 and 2006 on the specific query of type 1 DM debut of a tertiary hospital, with initial treatment assessment, at 1 and 5 years evolution.
Results
Patients were treated for newly diagnosed DM 1, 25 patients (72% male, 28% female) mean age 23.2 years (14–36), mean BMI: 23.18 (18–33.8%).The clinical presentation included: 88.24% presented with weight loss average (mean: 8.91 kg (4–18 kg), 28% of patients had ketoacidosis requiring hospitalization 41% (mean stay: 5.7 days).
In 65% of patients starting insulin therapy was prescribed in endocrinology. Of the patients who weren't initially treated in our Endocrinology Department 40% were treated with NPH, 20% with mixed insulin (rapid analogs and NPH) and 40% with OHA (oral hypoglicemic agents).
The initial insulin therapy regimens used in our clinic were: 5% 2 doses of NPH, 38.88% mixed insulin (NPH and rapid analog in three doses). 55.54% of patients initiated functional basal-bolus insulin therapy (Lantus/Levemir + rapid analogues).
At baseline, mean HbA1c: 10.58%, mean C-peptide: 1.1. Autoimmunity to GAD positive in 80%, 52% for IA2 and 23.8% for antiinsulin antibody.
After a year of evolution, 81.25% of patients have a functional basal-bolus insulin therapy, with a mean insulin requirements 0.49 U/kg per day, mean HbA1c 6.4%, average Peptide C: 1.0, Autoimmunity positive for GAD 79% for IA2 50% and 45.8% for anti-insulin Ab.
After 5 years of evolution, 100% of patients have a functional basal-bolus insulin therapy, with mean insulin requirements 0.75 U/kg per day, mean HbA1c 7.2%, with a mean weight gain of 1.9 kg and counting carbohydrate portions of up to 67% of cases.
Conclusions
In endocrinology tends to precocious intensive insulin therapy since DM1 debut, with an exponential increase in the usage of insulin analogues to mimic as much as possible the physiology of the pancreas. But there is a percentage that optimal treatment is delayed for late referral to the Endocrinology Department, mainly from primary care.

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P490

Assesment of diabetes knowledge evaluation in type 2 diabetic patients

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Introduction
Research has demonstrated that knowledge about medications, diet, exercise, home glucose monitoring, foot care, and treatment modifications is necessary to effectively self-manage diabetes, and the assessment of diabetes-related

knowledge is an important first step from which to individualize diabetes education programs and make evaluations of their effectiveness.
Purpose
To assess the knowledge of diabetes mellitus (about the disease, its risk factors, signs/symptoms, related complications and suitable diet and lifestyle) among our type 2 diabetic patients and to identify knowledge deficits and patient specific features that are associated with this knowledge.
Material and methods
A cross-sectional sample survey of patients' knowledge of diabetes was carried out by administering a 23-item diabetes knowledge questionnaire adapted to Spanish language to collect information. A total of 90 type 2 diabetic patients, mean age 62.1 (11.7 S.D.) attending our clinic during 4 months were interviewed. We collected data about age, sex, time of evolution, chronic complications, treatment, cardiovascular risk factors, comorbidities, anthropometric (weight, BMI, waist perimeter) and analytic parameters.
Results
Ninety patients were evaluated, mean age 62.1 ± 11.7 years, 57.1% men. Mean duration of the diabetes was 12 ± 8.66 years, with mean time of attendance in our Endocrinology Department 3.45 ± 3.47 years. 35.7% presented chronic complications disease-related; HTA was present in 70% of the patients, obesity in 73% and dyslipemia in 83.3%. Thirty percent of the patients were treated with oral hypoglycemic agents (OHA), 10% were insulin treated and 60% with combined treatment (insulin plus OHA).Mean BMI was 33.8 ± 4.8, and mean HbA1c 7.55 ± 1.05%. 51.9% of the patients answered correctly more than 75% of the questions and 48.1% of the patients more than 50%. None of the patients answered correctly <50%. The number of right answers was statistically significant regarding age (mean 58.8 ± 12.9 years in more than 75% of right answers vs 65.1 ± 10.1 years in 50–75% of right answers, *P* 0.05), but there were no significant differences depending on years of evolution (12.7 ± 10.2 vs 10.3 ± 6.3), BMI (33.5 ± 5.5 vs 33.7 ± 4.9), HbA1c (7.4 ± 1.2 vs 7.8 ± 0.7%) or time of endocrinology clinic attendance (4 ± 4.1 vs 2.7 ± 2.5 years). There were also no statistically significant differences depending on sex, chronic complications or previous educational programms. Insulin treated patients answered correctly more questions (more than 75% of right questions) than patients treated with OHA (100% in insulin treated patients vs 41.2% en patients treated with OHA *P* 0.026).
Conclusions
In our group of DM2 patients, we can conclude that most of them had appropriate diabetes related Kwowledge. Patients with better scores had less mean age, and were treated with insulin.
Priority needs to be given by education programmes to give patients a better knowledge of their disease, to prevent premature morbidity and mortality associated with diabetes.

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P491

Advantages of using DPP-4 inhibitors in patients with type 2 diabetes mellitus and overweight

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Objective
Analyze the use of DPP-4 inhibitors (iDPP4) in a specialized consultation as well as metabolic impact, weight and safety in patients with diabetes mellitus type 2 (DM2).
Material and methods
We analyzed 100 patients with DM2, derivative to Diabetes hospital with debut or poor glycemic control. We initiated them sitagliptin 100 mg/day and reinforced Diabetes education. We studied the HbA1c, weight and severe hypoglycemia. Statistical analysis was performed with SPSSv18, making a *t*-student and Wilconson test.
Results
Forty-six percent of our patients were referred in debut and the remaining 64% with DM2 poorly controlled. They had a mean age of 57.6 ± 12.7 years and a mean of 4.8 ± 6.4 years of DM2 evolution and had a mean BMI of 30.5 ± 4.9 kg/m^2. We appreciated a total decrease of mean HbA1c of 9.2 to 6.6% (−2.7*%) and a total mean weight loss of −1.57* kg. In none of the patients observed severe hypoglycemia. Besides the subgroup analyzed by combined use with sitagliptin.
– Monotherapy (n=3): decrease mean HbA1c from 7.3 to 6.1% (−1.2%*), and average weight loss of −2.80 kg*.
– Dual therapy (n=55): decrease mean HbA1c from 9.4 to 6.4% (−3.0%*), and average weight loss of −2.97 kg*.
– Triple therapy (n=7): decrease mean HbA1c from 8.1 to 6.9% (−1.2%*), and average weight loss of −3.09 kg*.
– Insulin basal therapy (n=35): decrease mean HbA1c from 9.4 to 6.6% (−2.8%*), and average weight loss of −0.75 kg*.
*P<0.05
Conclusions
The most frequent use of iDPP4 was performed in combination therapy with metformin, followed by association with basal insulin plus metformin. Therapy with DPP-4 is effective treatment option in the first step (in intolerant to metformin), as 2nd oral drug, in triple therapy, even with basal insulin. The profile beneficial weight and hypoglycemia security makes it ideal for the treatment of overweight patients with DM2.

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P492

Hypoglycemic evaluation of *Swietenia macrophylla* King combined seed and endocarp extracts: a comparative study between hot water extraction and aqueous-methanol extraction

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Solvent and process variables were critical to optimize the extractions of potential hypoglycemic compounds within medicinal plants. Hence, the undertaken study was designed to evaluate the hypoglycemic effectiveness exhibited by extraction performed in decoction manner using water as solvent and compares it with an aqueous-methanol solvent extraction. To evaluate the extractions prepared, phytochemical assays and *in vivo* study in induced-diabetic rats were carried out. Results indicates aqueous-methanol extract as active for alkaloids, flavonoids, cardiac glycosides, reducing sugar, saponins, tannins and terpenoids. It is also more efficient than the hot water extract in improving altered biological parameters impacted by hyperglycemia. As such, aqueous-methanol appears ideal for extracting the necessary potent hypoglycemic compounds within the plant parts studied.
Key Words
S. macrophylla King, Hot Water Extract, Aqueous-Methanol Extract, Hypoglycemic.

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P493

Evaluation of BICI therapy in pregestational type 1 diabetes

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Objective
To evaluate in our area BICI therapy efficacy in pregestational type 1 diabetes.
Methods
We conducted a descriptive retrospective analysis studying metabolic control and obstetrics results in patients with pregestational type 1 diabetes, treated with BICI between 2004 and 2012 in our hospital.
Results
We evaluated 28 patients with type 1 diabetes and diabetes duration of 17.3 ± 6.6 years. In 27.3% of patients the treatment with BICI was indicated for reasons other than prenancy. Of the remaining, 18.7% performed treatment with BICI planning pregnancy, and in 81.3% started this therapy for bad metabolic control during the pregnancy, despite intensive insuline therapy. Only 39.3% of pregnancies were planned. Women had no ketoacidosis or severe hypoglycemia. Regarding the onset and progression of chronic complications, only in one patient was detected progression of diabetic retinopathy. The completion of gestation was 34.2 ± 5.4 weeks and was performed by planned cesarean in 28.6%, urgent cesarean in 25% and vaginal delivery in 14.3% of the cases. Two patients had spontaneous abortion. There were no others perinatal complications, presenting fetal macrosomia 31.25% of newborns.
Conclusions
BICI therapy represents a safe and effective strategy to optimize metabolic control in patients with pregestational type 1 diabetes poorly controlled with

conventional intensive insulin treatment, to plan pregnancy or to achieve good metabolic control in the case of unplanned pregnancies.

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P494

Quality indicator of glycemic status in patients with diabetes mellitus in primary health care in the Kyrgyz Republic

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Introduction
Basic medical care to patients with diabetes mellitus (DM) in Kyrgyzstan is on the primary care level, in family medicine centers.
Methods
We have analyzed, in a random order histories of diseases of 928 outpatients observed and registered patients with diabetes in primary health care clinics, in Bishkek.
Results
The basic amount of patients were women 656 (70.7%), and men 272 (29.3%) respectively. The mean age was 63.8 ($\pm$10.5) in range 29–89 years. Glycemic status of patients was determined by following tests: fasting glucose, glucose 2 h after eating, and glycated hemoglobin (HbA1c). Examination of blood glucose was performed in all patients of our study after getting informed consent. The result of our study showed the mean value of glucose 8.71 mmol/l ($\pm$3.04), where we discovered 318 (34.3%) compensated patients vs 610 (65.7%) decompensated patients. Situation of blood glucose evaluation after a meal and HbA1c of patients with diabetes mellitus in Kyrgyzstan is in a poor condition, as primary care physicians do not pay enough attention to this analysis. In result of our investigation there were evaluated only 82 (8.84%) patients by blood glucose test after meal: which showed 5 (6.1%) – compensated and 77 (93.9%) – decompensated. No less important analysis is the evaluation of HbA1c which was examined only in 222 (23.9%) patients, with mean value of HbA1c – 8.44% ($\pm$3.7%), where we revealed 58 (26.1%) compensated patients vs 164 (73.9%) decompensated.
Conclusions
Thus, my results proved that the quality of primary care of diabetic patients in Kyrgyzstan requires improvement and perfection. It is recommended to introduce clear standards of diagnostic services.

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P495

Pre-diabetes study in Libya: delay and prevention T2DM

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T2DM is expected to rise to 552 million by 2030. DM is associated with complications which result in reduced quality of life and premature mortality.
T2DM has a long asymptomatic pre-clinical phase ($\sim$5–10 years) which frequently goes undetected. Benefits of early diagnosis on outcomes in screen-detected DM remain unclear. Some indirect evidence suggesting that early detection and intervention may be beneficial.
Screening for DM will identify individuals with intermediate hyperglycemia (IGT and IFG) who may benefit from interventions to prevent or delay DM and complications. Lifestyle changes can prevent or delay DM by up to 58% in people with prediabetes.
Libya has 6 million inhabitants with $\sim$17% people with DM and about 24% if we add people with IFG in 2009. This is the first study in Libya screening for people with pre-diabetes.
Study aims: i) establishing screening program to prevent or delay DM. ii) identifying people at high risk of DM and with asymptomatic hyperglycemia. iii) encouraging people to have a risk assessment.
Four thousand non-diabetic individuals in the capital city of Tripoli (2.2 million inhabitants), age 45 years and above with conditions that increase risk of DM will be included. Risk assessment questionnaire followed by diagnostic blood tests will be used.
Venous blood tests will be offered to adult with high risk scores. If FBS $<$100 mg/dl or HbA1c of $<$6.0%, a brief intervention and weight loss program will be offered. High risk score and FBS of 100–126 mg/dl or HbA1c of 6.0–6.4%, information will be offered in their particular risk factors. People with possible T2DM, FBS of 126 mg/dl or above, or HbA1c of 6.5% or above, but no symptoms of T2DM, second blood test will be carried if type 2 diabetes is confirmed treatment will be offered.

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P496

Molecular docking research analysis of novel natural and synthetic PTP 1B inhibitors as potential therapeutic target for diabetes mellitus

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Augmented pervasiveness of type 2 diabetes mellitus and obesity has amplified the medical necessitate for new agents to treat these disease states. Both type 2 diabetes and obesity are connected to the resistance to the hormones insulin and leptin. Protein tyrosine phosphatase 1B (PTP1B) has been shown to function as negative regulator of insulin signalling as well as leptin signal transduction. At present there are copious compounds synthesized as PTP1B inhibitors. The development of compound libraries with more selective PTP1B inhibitors has been bumped up by the realization that many natural products have PTP1B inhibitory activity and therefore are attention-grabbing biologically lead compounds. This research exertion shows the molecular docking analysis of novel synthetically prepared compounds and new-fangled isolated natural PTP 1B inhibitors as novel target for type 2 diabetes.

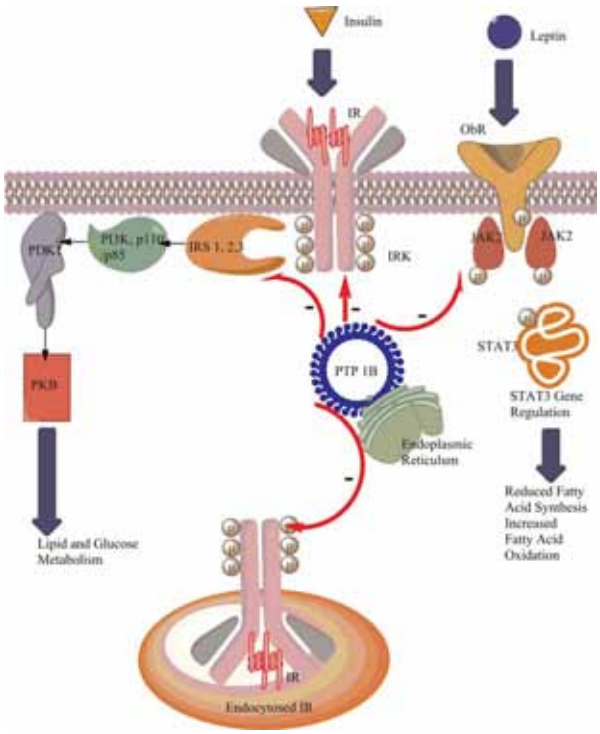

Figure 1 Negative Regulation of Insulin and Leptin Pathways by PTP 1B

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Endocrine disruptors

P497

Reproductive aging in rats is altered by developmental exposure to mixtures of endocrine disrupters

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Long-lasting and/or delayed reproductive effects of developmental exposure to mixtures of environmental chemicals were investigated in rats. Wistar rats were exposed to mixtures of estrogenic or anti-androgenic endocrine disrupters in pregnancy and lactation, and effects in offspring at 12–18 months of age were studied. The mixture included 13 estrogenic and anti-androgenic chemicals, including phthalates, pesticides, u.v.-filters, bisphenol A, butylparaben and paracetamol, and the mixture ratio was chosen to reflect high end human intakes. In the animal study, two groups received exposures of 150 and 450 times high end human intake levels of these chemicals combined, and one group received a mixture including only the anti-androgens.
Females exposed to the mixture showed earlier reproductive aging, and males had reduced epididymal sperm count, histological changes in the epididymis, and normal prostatic aging (atrophy) was altered towards hyperplasia. A possible increased incidence of pituitary adenomas in the mixture groups could be related to the altered age of reproductive senescence.These effects were mainly attributed to the anti-androgenic compounds in the mixture, as comparable effects were in most cases seen in animals exposed to the total mixture and in the group receiving a mixture of anti-androgens only. A previous publication describes endocrine disrupting in prepubertal offspring (Christiansen *et al.*. *Int J Androl* 2012 **35** (3) 303–316), and these early findings can be a signal of severe adverse effects later in life.
This study demonstrated that developmental exposure of rats to endocrine disrupters can induce long-lasting effects manifested in early reproductive senescence, and perinatal programming of the hypothalamo-pituitary–gonadal axis may account for the observed changes in the timing of aging-related events.

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P498

An investigation about metabolic disruption: organostannic compounds as PPAR gamma agonists

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The parallel increase in the amount of environmental contaminants and in the prevalence of some human diseases has led to a growing interest in understanding how these compounds, so-called endocrine disruptors, may affect human health. In this scenario, the concept that obesity is the result of the interplay of genetic and lifestyle factors has been changing due to the growing evidence that environmental contaminants might alter endocrine function.
Organostannics are a distinct class of organic pollutants related to paints used in overseas transports and agrotoxics, and have emerged as potential metabolic disruptors due to their effects on nuclear receptors. Tributyltin (TBT) was the organostannic compound studied and has been shown to be a strong agonist for peroxisome proliferator-activated receptor-γ (PPARγ) and stimulate adipogenesis, with the potential to induce weight gain and other unfavorable effects of strong PPARγ activation. However, little is known about the effect of other organostannic compounds on this receptor.
In this study, we describe the agonist effects of the organostannic pollutants dibutyltin dilaurate (DBT) and benzoate TBT on PPARγ in HeLA and human mesangial cells cotransfected with PPARγ-GAL4 and the UAS-tk-luc reporter. This effect appeared to be specific for PPARγ, since DBT, benzoate TBT and also TBT had no activity on glucocorticoid, estrogen and thyroid hormone receptors when assessed by reporter gene assays using the respective expression vectors and responsive element-driven luciferase reporter.
Further studies are being carried out to investigate the effect of these compounds on cell-based adipogenesis, PPARγ-responsive genes and also *in vivo*. Additionally, we seek to investigate their mode of binding to the binding pocket of PPARγ. These results may provide a better understanding of how these pollutants affect in human health and possibly the increasing rates of obesity and its associated diseases worldwide.

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P499

Tributyltin changes the thyroid gland morphology of male rats

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Triorganotins, such as tributyltin (TBT), are environmental contaminants commonly found in antifouling paints that are used on the ships and other vessels. Unfortunately, these chemical are also suspected to cause endocrine-disrupting effects in mammals, due in part to their possible transfer through marine food chains and to the consumption of contaminated seafood. The importance of triorganotins as environmental endocrine disruptors in different animal models is well known; however, the adverse effects on thyroid gland are less well understood. We evaluate the potential histophysiology changes induced by TBT on the thyroid gland. Male Wistar rats (8 weeks old, ±250 g) were divided into 3 groups: control (vehicle, 0.4% ethanol), TBT_1 ($100\ ng\ kg^{-1}\ day^{-1}$) and TBT_2 ($200\ ng\ kg^{-1}\ day^{-1}$) treated for 15 days by gavage. After the treatments, the animals were sacrificed and thyroid glands were fixed for histological analysis. TBT (both doses) promoted a disorganization of follicular cell groups, with hypertrophy and hyperplasia of thyrocytes, elevated number of mast cells, increase in collagen deposition and glandular congestion compared with control thyroid gland. However, we did not observe changes in plasma levels of T_3 and T_4 after 15 days of treatment. Also, we showed a significant decrease in cell viability with the doses of 2.0×10^{-1} and 1.0×10^{-1}ng/ml TBT for 2 and 3 h, using PCCL3 cells (*in vitro* model of thyrocytes). In this work, we observed that TBT-exposure in thyroid gland induces morphological changes and may correspond to a potential risk factor for thyroid disorders in mammals.

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P500

Sex hormone-induced gender differences in vascular muscle cells motility are susceptible to the environmental disruptors

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Sex steroid hormone-induced variations of vascular smooth muscle cells (VSMCs) migration are critical in determining the sex/gender-related differences in male and female pathophysiology of cardiovascular system. Although several substances present in the environment, defined endocrine disruptors (EDs), could interfere with androgen and estrogen effects, the sex/gender-related susceptibility of VSMC motility to these substances is completely unknown. Here, naringenin (Nar) and bisphenol A (BPA) effects on male and female VSMC motility has been evaluated. VSMC motility was determined by Wound healing and cell migration assays. 17β-estradiol (E_2, 1–10 nM) induced a dose-dependent inhibition of motility in female-derived VSMC. In contrast, neither testosterone nor dihydrotestosterone (0.01–100 nM) modified male-derived VSMC motility. ERβ subtype-dependent activation of p38 was necessary for the E_2 effect on cell motility. High BPA concentration prevented E_2 effects in female-derived cells being without any effect in male-derived cells. Nar mimicked E_2 effects on female-derived cells even in the presence of E_2 or BPA or in male-derived VSMC. This latter effect was blocked by ERβ subtype inhibitor pre-treatment, but not by the AR inhibitor. These observations indicate that ERβ plays a pivotal role in vasculoprotection being the main receptor to mediate the effects of E_2 in the vascular wall. Moreover, although E_2 signals in VSMC are more susceptible to the impact of EDs than androgen signals, male-derived cells, expressing both ERs, possess a similar susceptibility to EDs than female-derived cells.

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P501

Vitamin E protects against methomyl-induced reproductive toxicity in pregnant female rats

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Purpose
The aim of this study was to evaluate the effects of methomyl 25%WP (Lannate, carbamate broad spectrum insecticide) on the reproductive parameters in female Wistar rats during pregnancy and the possible protective role of vitamin E against methomyl-induced toxicity.
Methods
The animals were randomly divided into four groups, eight of each. Group I served as control; Group II received orally a dose of methomyl (Met, 10 mg/kg per day), Group III was dosed via gavage by vitamin E (Vit E, 100 mg/kg per day), Group IV received a concomitantly treatment of methomyl plus vitamin E (Met + Vit E). The treatment lasted from GD1 to GD18. Body weight gain, food and water intake, progesterone levels (at GD18) and pregnancy outcomes were evaluated, then after post-partum, dams were sacrificed, adrenal and ovary organs were excised, weighed and used for histological examinations.
Results
When compared with control, methomyl group showed a significant decrease in ovary relative weights, progesterone levels, litter size, sex ratio, gestation index and the newborn mean weight. In the other hand, the histological adrenal and ovary examinations revealed pathohistological changes marked by cells disorganization and vacuolization in the adrenal gland, as well as degenerative changes with a few number of healthy follicles and many atretic folliclesat different stages of development in ovary. In addition, Vit E group displays similar pregnancy outcomes and histological structure in both adrenal and ovary organs when compared with control, however a slight elevation in progesterone level was noted. The co-administration of Vit E along with methomyl reversed partially or completely all the adverse toxic effects generated by methomyl. In conclusion, it is clear that vitamin E has a potential protective role against methomyl-induced reprotoxicity in female treated during pregnancy and this may result from its antioxidant properties proven in several recent studies.

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P502

Bisphenol-A modulates proliferation of human breast adenocarcinoma cells (MCF-7) by modulating apoptosis and cyclin-A

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Endocrine disruptors represent exogenous substances that alter hormonal, reproductive, immune and homeostatic systems. Bisphenol-A (BPA) in the form of polymer is a part of polycarbonates used in plastics, food packing and medical devices. Incomplete BPA polymerization and cleavage of weak chemical bonds result in monomer release/leakage to the foods and beverages. Many studies have associated exposure to BPA with higher incidence of hormone-dependent mammary and prostate carcinomas. In present study we investigated the effect of BPA on proliferation of MCF-7 cells in cell culture. Dose (1×10^{-6}–1×10^{-12}M) and time (24–120 h) dependent effects of BPA were compared with estradiol (E2) effects. Cell growth was measured by WST-1 test, *de novo* synthesis of DNA with BrdU incorporation to DNA, apoptotic proteins and cyclin-A were determined by Western blot. Compared to E2, high concentrations of BPA significantly stimulated cell growth after 48 h treatment. Long-term effect of BPA (96, 120 h) similarly stimulated cell proliferation (40%), however the impact did not achieve the effect of E2 (80–130%). Stimulatory effect of BPA on cell proliferation (48 h) was accompanied by huge increase (~200%) of *de novo* DNA synthesis even after 24 h. At longer time expositions BPA effects significantly declined and were comparable with those of E2. BPA reduced expression of pro-apoptotic proteins p53 (48 h) and Bax (24 h) by dose dependent manner (vs E2), while expression of anti-apoptotic protein Bcl-2 was increased after 96 h only. BPA in low and high doses significantly reduced cyclin A protein expression as compared to E2. In conclusion, BPA effects on MCF-7 cells proliferation result from alteration of several cellular processes like synthesis DNA, disturbed synthesis/degradation of apoptotic proteins and proteins of cell cycle division and replication. Effects of BPA are dose-dependent.
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P503

Protective potential of 17-beta-estradiol on membrane linked functions in aging female rats: a behavioral, biochemical and ultrastructural study

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Background
Alzheimer's disease (AD) is the most common form of dementia in the elderly. AD is characterized by the presence of amyloid plaques which are formed from deposits of b-amyloid protein (Ab). These changes increase during menopausal condition in females when the level of estradiol is decreased.
Objective
The objective of this study was to investigate neuroprotective potential of 17β estradiol (E2) treatment on the activities of acetylcholinesterase and monoamine oxidase, membrane fluidity, neurolipofuscin, genomic DNA degradation, protein oxidation levels and testing learning memory, occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol.
Methods
The aged rats (12 and 24 months old) ($n=8$ for each group) were given subcutaneous injection of 17β -estradiol (0.1 μg/g body weight) daily for 1 month. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and brains were isolated for further study. Learning was tested in a Morris water maze and ultrastructural studies of brain region by MRI.
Results
The results obtained in the present work revealed that normal aging was associated with significant increases in the activity of monoamine oxidase, genomic DNA degradation and protein oxidation levels in the brains of aging female rats, and a decrease in acetylcholinesterase activity and membrane polarization. Ultrastructural studies of the frontal cortex of exposed rats revealed that the changes were more pronounced in the aged treated rats in terms of presence of lipofuscin, vacuolization and lysosomal degradation. Our data showed that exogenous administration of E2 brought these changes to near normalcy in aging female rats.
Conclusions
It can therefore be concluded that E2's beneficial effects seemed to arise from its, antioxidant and antilipidperoxidative effects, implying a therapeutic potential drug for age related changes. Based on our studies and others, we conclude that E2 have therapeutic potential for adjunctive therapy for the AD.

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P504

Effects of chlorobenzenes on the histamine (HA) activated arginine-vasopressin (AVP) release – from rat pituitary cells

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Introduction
The endocrine disruptor compounds (EDC), as persistent organic pollutant (POP) agents, have ability to interfere with endocrine systems. These properties of EDC may modify the homeostasis of biological organisms.
Aim
The aim was to investigate the effects of chlorobenzenes (ClB), hexa-chlorobenzenes + 2,4,6,trichloro-benzenes, with chronic exposure and extremely low doses on histamine mediated neuroendocrine functions.
Methods
The model-animals, Wistar male rats (bw: 80–200 g), were exposed to ClB (1.0 μg/bw kg per day) via a gastric tube for 0, 30, 60, and 90 days. After the ClB treatment, organs and bodyweight, furthermore the hepatic transferase enzymes

of Wistar rats were measured. At the completion of the *in vivo* treatment plasma AVP was determined by RIA, and pituicytes from the rat neurohypophyses were cultured. HA-stimulated AVP release was investigated in the cell cultures. The statistical analyses were performed by ANOVA.
Results
AVP secretion was increased by HA stimulation as compared to control (124.57 ± 1.39 pg AVP/mg prot) vs (43.29 ± 1.39 pg AVP/mg prot). These AVP results demonstrate that the neuro-endocrine function was significantly modulated by a chronic and subtoxical ClB treatment.
Conclusion
It can be hypothesised that ClBs may influence homeostatic processes of exposed animals through endocrine chanels.

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P505

Rise of obesity incidence in ChNPP accident survivors is related to abnormal secretion of α-melanotropin

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Introduction
Accident at the Chernobyl NPP (ChNPP) in Ukraine on the 26th of April, 1986 was followed by the intensive release of a wide range of radioactive elements with affinity to many endocrine tissues. The mentioned radioactive fallout resulted in both internal and external radiation exposure, among others, of the central endocrine structures of the human brain.
Methods
We have conducted a retrospective analysis (20–25 years upon radiation exposure) of anthropometric indices in 606 survivors of the ChNPP accident and in 589 not exposed persons from the general population of Ukraine (i.e. control group). Basal serum levels of α-melanocyte-stimulating hormone (α-MSH), leptin and some other hormones were assayed in 100 of study subjects.
Results
Higher incidence of borderline obesity – 37% ($\chi^2_{Yates}=4.22$, $P=0.04$) and of primary obesity – 32.5% ($\chi^2_{Yates}=8.5$, $P=0.004$) was found in the ChNPP accident survivors vs persons in the control group (31.1 and 24.6% respectively). Normal body mass was more prevalent in the general population (39.3%; $P=0.002$). The subgroup analysis indicated that primary obesity was more often found in ChNPP accident emergency workers of the so-called 'non-iodine period' of 1986–1987 (47.3%) and of 'iodine period' of 1986 (34.2%). For the first time there was revealed a new abnormal way of a reaction on radiation namely – the 'blunted' protective response of the physiological increase of α-MSH secretion along with body mass index elevation normally preventing further growth of adipose tissue. There is no increase of α-MSH secretion or even there is a hormone deficiency in most survivors of the ChNPP accident having borderline obesity or obesity.
Conclusions
Received data indicate to the increased risk of borderline obesity and obesity after the prolonged exposure to radiation in moderate doses. The mentioned risk is stipulated by disorders in melanocortin system resulting in α-MSH deficiency at the background of obesity that can be considered as a marker of such an abnormality.

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P506

Endocrine functions in premenopausal and postmenopausal women

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Introduction
The anabolic hormones, namely GH, testosterone and dehydroepiandrosterone, as well as the reproductive function of women, enter a period of progressive insufficiency which is characteristically designated as somatopause, andropause and adrenopause respectively. The aim of the study was to compare the endocrine functions between premenopausal and postmenopausal women.
Subjects and methods
The study was included 40 premenopausal (mean age 34.9 ± 4.8 years) and 40 post menopasal women (mean age 65.1 ± 8.6 years) GH, free thyroxine (FT4), free triidotironin, TSH, adrenocorticortropichormone (ACTH), cortisol (COR), dehydoepiandrosteronesulfat (DHEA-S), FSH, LH, prolactin (PRL), testosterone, estradiol (E2) and fasting insulin levels were measured in all patients.
Results
Mean levels of FSH ($P=0.04$), LH ($P=0.02$), COR ($P=0.01$), ACTH ($P<0.001$) and fasting insulin ($P=0.02$) in post menopausal were higher than that of premenopausal women. However, E2 ($P=0.04$), TSH ($P=0.01$), FT4 ($P=0.001$), PRL ($P=0.05$) and DHEA-S ($P=0.01$) levels were lower in post menopausal than premenopausal women.
Conclusion
The study demonstrated that E2, TSH, FT4, PRL and DHEA-S levels were decreased with aging.

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P507

Peutz Jegher syndrome with multiple endocrinal failures

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Introduction
Peutz-Jeghers syndrome (PJS) is a rare familial disorder with an incidence of one in 12–30,000 live births characterized by mucocutaneous pigmentation, gastrointestinal and extra gastrointestinal hamartomatous polyps and an increased risk of malignancy.
Case presentation
We report a 22-year-old female hypothyroid since age of 4, type 1 diabetic since age of 11, who presented with melaena. Pigmentation of the buccal mucosa, multiple lipomatosis, vitamin D deficiency. Upper endoscopy, colonoscopy, enteroscope revealed multiple polyps. In stomach pyloric ring, rectum biopsy showing hamartomatous polyp.
Conclusion
A case of peutz Jeghers syndrome with multiple lipomatosis, she is type 1 diabetic with early childhood hypothyroidism, vitamin D deficiency. These combinations of different aetiologies in the same patient might raise the suspension of a new syndrome waiting for other observational studies.
Keywords: peutz-Jeghers syndrome; type 1 diabetes; hypothyroidism; multiple lipomatosis

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Endocrine tumours and neoplasia

P508

Heriditary forms of medullary carcinoma of the thyroid: about a new family

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The medullary carcinoma of thyroid is rare and represent 5–10% of the thyroid cancers. It appears under sporadic and family forms in more than a third of cases. It becomes integrated them into the multiple endocrine neoplasms type 2 of autosomique dominant transmission associate with germinal mutations of the gene RET. We bring report in this study, the observation of a new family shape. BL index case was diagnosed in the age of 42 years further to the appearance of a severe HTA associated with renal colics and a goiter. The paraclinic assessment reveals a bilateral pheochromocytoma, a medullary carcinoma and a primary hyperparathyroidism. The molecular study of RET gene reveals a germinal mutation of C634 R (TGC/CGC) exon 11. Bilateral surrenalectomy is realized followed by a thyroidectomy and parathyroidectomy. The evolution is marked by sudden death of the patient with recurrence of pheochromocytoma. A family investigation was indicated. Unfortunately, only eight subjects agreed to submit themselves to the exploration. All were affected and presented the same paintings phenotype and genotype. Prevalency of the medullary

carcinoma family of novo is estimated between 5 and 16% of cases. So molecular analyses of gene RET must be realized systematically. This allows a early diagnosis and a specific and preventive care. 0.634 mutations is the most frequent and finds itself in more than two thirds of the cases with a correlation phenotype genotype. The risk of pheochromocytoma, hyperparathyroidism and aggressiveness of tumors are higher when patients are affected by this mutations.

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P509

Abstract unavailable.

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P510

Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993

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Introduction
Adrenocortical carcinoma (ACC) has a reported annual incidence of 0.5–2.0 cases per million individuals. Updated population-based studies on incidence are lacking. The aim of this nationwide survey was to describe the incidence and survival rate of ACC in the Netherlands. Secondary objectives were to evaluate changes in survival rates and in the number of patients undergoing surgery.
Methods
All ACC patients registered in the Netherlands Cancer Registry between 1993 and 2010 were included. Data on demographics, stage of disease, primary treatment modality and survival were used.
Results
Included were 359 patients, of whom 196 were female (55%). Median age at diagnosis was 56 (range 1–91) years. The 5-year age-standardized incidence rate decreased from 1.3 to 1.0 per one million person-years. Median survival for patients with stage I–II, stage III and stage IV was 159 months (95% CI 93–225 months), 26 months (95% CI 4–49 months) and 5 months (95% CI 2–8 months) respectively ($P<0.001$). Improvement in survival was not observed, as reflected by the lack of association between survival and time of diagnosis. The percentage of patients receiving treatment increased significantly from 76% in 1993–1998 to 88% in 2005–2010 ($P=0.047$), mainly explained by an increase in surgery in stage III–IV patients.
Conclusion
These nationwide data provide an up to date description of ACC epidemiology in the Netherlands. A trend towards a decreasing overall incidence rate was observed. Survival rates did not change during this period despite an increased number of surgical procedures.

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P511

Investigation of novel chemotherapeutic combinations in a tumor model for adrenocortical carcinoma

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Medical treatment of adrenocortical carcinoma (ACC) is limited to common cytotoxic agents, which are usually given in combination with mitotane (M). Recently, we investigated together with M the effects of i) the classical EDP protocol (etoposide, doxorubicin, and cisplatin) and ii) a novel paclitaxel containing scheme PDP (paclitaxel, doxorubicin, and cisplatin) on human NCIh295 cells indicating anti-tumoral superiority of PDP-M over EDP-M regarding cell viability ($P=0.001$), apoptosis ($P=0.001$) and proliferation ($P<0.01$). Since we found furthermore evidence for an extraordinary uptake phenomenon of liposomes by ACC cells we included for consecutive *in vivo* experiments liposomal variants called LEDP-M (etoposide, liposomal doxorubicin, and liposomal cisplatin) and LPDP-M (nab-paclitaxel, liposomal doxorubicin, and liposomal cisplatin). In short-term therapeutic experiments on NCIh295-xenografts EDP-M did not induce a significant loss of tumor cells while PDP-M ($P<0.01$), LEDP-M ($P<0.01$) and LPDP-M ($P<0.01$) resulted overall in significant tumor cell reduction compared with controls. LEDP-M ($P<0.01$) and LPDP-M ($P<0.05$) induced furthermore apoptosis as quantified by TUNEL staining. Similar effects were detectable with TUNEL on patient's ACC-xenografts comparing LPDP-M with PDP-M ($P<0.05$), but not between EDP-M and LEDP-M. Blood counts of PDP-M and LPDP-M treated mice showed a tendency to leukocyte reduction without statistical significance vs controls, while EDP-M and LEDP-M treatments lead to leucopenia ($P<0.01$). HE-stained kidneys from LEDP-M and LPDP-M treated mice appeared unaffected compared with controls, while kidneys of EDP-M and PDP-M groups showed by trend reduced nuclear staining intensities and more diffuse cell borders. Long-term experiments on NCIh295-xenografts revealed highest and sustained anti-tumoral effects for LEDP-M. Beside significant differences in tumor sizes between controls and LEDP-M, we detected beginning from day 35 ($P<0.05$) up to day 53 (end of experiment, $P<0.001$) highly significant reduced tumor sizes for LEDP-M compared with EDP-M. In summary, liposomal chemotherapies could represent promising approaches that would deserve testing in clinical protocols for patients with ACC.

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P512

Biochemical diagnosis of pheochromocytoma using plasma free normetanephrine, metanephrine and methoxytyramine: importance of supine sampling under fasting conditions

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Objective
To document influences of sampling of blood under supine fasting versus seated non-fasting conditions on diagnosis of pheochromocytoma using plasma concentrations of normetanephrine, metanephrine and methoxytyramine (P-NMN/MN/MTY).
Design and methods
P-NMN/MN/MTY were measured by liquid chromatography with tandem mass spectrometry in 695 patients at five centers, two of which complied with requirements for supine sampling after an overnight fast and three of which did not. Pheochromocytomas were found in 109 patients (60 non-compliant, 49 compliant) and not in 586 patients (144 non-compliant, 442 compliant).
Results
P-NMN/MN/MTY in patients with pheochromocytoma did not differ under compliant and non-compliant conditions. However, sampling under non-compliant compared to compliant conditions resulted in 50 and 40% higher ($P<0.001$) median plasma concentrations of NMN (84 vs 56 pg/ml) and MTY (17.3 vs 12.4 pg/ml) in patients without tumors. Upper cutoffs were also substantially higher under non-compliant compared to compliant conditions for both NMN (241 vs 144 pg/ml) and MTY (62 vs 33 pg/ml). Differences for MN were minimal. Use of upper-cut-offs calculated for non-compliant compared to compliant conditions resulted in substantially decreased diagnostic sensitivity (88.1 vs 98.2%). Use of upper-cut-offs calculated for compliant conditions was

associated with a diagnostic specificity of only 78.5% for sampling under non-compliant conditions compared to 94.8% with compliant conditions.
Conclusions
High diagnostic sensitivity of P-NMN/MN/MTY for detection of PPGLs can only be guaranteed using upper-cut-offs of reference intervals appropriately calculated under supine fasting conditions. With appropriately estimated upper-cut-offs, sampling under seated non-fasting conditions can lead to a fivefold increase in false-positive results necessitating repeat sampling under supine fasting conditions.

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P513

The embryonic transcription factor *TBX1* is expressed in adult parathyroid cells and might be involved in parathyroid tumorigenesis

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Cancer cells and embryonic stem cells share many key biological properties and have common genetic signature: transcription factors regulating self-renewal and differentiation have been found expressed in human cancer cells. We focused our attention on *TBX1*, the gene involved in 22q11.2 microdeletion/DiGeorge syndrome, which is involved in heart, thymic and parathyroid cells differentiation.
Real-time PCR, western blot and immunohistochemistry demonstrated that *TBX1* mRNA and protein were expressed in normal parathyroid glands ($n=3$). Immunofluorescence (IF) showed a positive staining in parathyroid PTH+ cells with a cytoplasmic and nuclear localization. Typical parathyroid adenomas ($n=23$) expressed TBX1 at higher levels than normal glands. The regulation and the role of *TBX1* gene in parathyroid adenomas were further investigated. Functional studies were performed in HEK293 cells, since they expressed TBX1 mRNA and protein. We tested the hypothesis that *TBX1* expression might be regulated by signalling pathway involved in embryogenesis such as BMP/SMAD and Wnt/β-catenin. Treatment for 3–16 hours with 20 ng/ml BMP4 increased *TBX1* mRNA levels. Interestingly, we found by IF that tumoral parathyroid cells expressed the BMP4 receptor BMPR1A. By contrast, *TBX1* mRNA levels were inhibited by β-catenin accumulation induced by 8-hours of treatment with 10–20 nM lithium chloride. Furthermore, the activation of the calcium sensing receptor (CaSR) by stimulating for 24 hours HEK293 cells stably transfected with the human CaSR, with increasing calcium as well as R568 concentrations, the CaSR agonist cinacalcet, induced a reduction in *TBX1* mRNA levels. Silencing of *TBX1* gene in both HEK293 and tumoral parathyroid cells induced a significant reduction in *TBX1* target genes such as *WNT5a* and BMP4-induced increase in inhibitor of differentiation-1 (*Id1*) mRNA expression levels. In conclusion, an embryonic signature has been identified in adult parathyroid cells and it is suggested to be involved in parathyroid tumorigenesis. New potential therapeutic targets for parathyroid tumours have been discovered.

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P514

Characterization and sub-cellular localization of somatostatin receptors in DU-145 and PC-3 human androgen-independent prostate cancer cells: effect of mono- and bi-specific somatostatin analogs on cell growth

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Somatostatin (SRIF) is an inhibitory hormone that plays a regulatory role in several cell functions including cell proliferation. SRIF acts through five specific membrane receptors (sst1-5), expressed on SRIF-target cells. SRIF and ssts may play a significant role in the progression and neuroendocrine differentiation of human prostate cancer (PCa). However, conflicting results have been reported in the literature on ssts heterogeneity and specific cell localization in PCa. Aim of this study was to evaluate in two androgen-independent human PCa cell lines DU-145 and PC-3 cells: (a) the gene and protein expression of ssts; (b) the sub-cellular localization of the different ssts; (c) the effects of new mono- and bispecific SRIF analogs (SSAs) on cell proliferation and activation of proteins involved in the regulation of the cell cycle; (d) the constitutive and SSAs-driven ssts dimerization. DU-145 and PC-3 cells express all SRIF receptors at both gene (SSTR1-5) and protein (sst1-5) levels. Moreover, sst1/sst2 and sst2/sst5 receptor dimers were constitutively present at the cell membrane. A 48 h treatment with BIM-23704 (sst1/sst2) and BIM-23244 (sst2/ss5) compounds increased the amount of sst1/sst2 and sst2/sst5 dimers, respectively. Sub-cellular organelle separation of cell lysates showed a different sst1, sst2 and sst5 nuclear, lysosomal and microsomal distribution, according to the different recycling dynamics of these isoforms. In DU-145 and PC-3 cells, a 48 h treatment with BIM-23244 (sst2/5) and BIM-23926 (sst1) analogs were more effective in inhibiting cell proliferation (-30%) in the dose-range tested (10^{-10}–10^{-6} M), compared to BIM-23120 (sst2), BIM-23206 (sst5) and BIM-23704 (sst1/sst2) compounds. Moreover, in DU-145 cells BIM-23244 and BIM-23926 activated p21 and phosphorylated ERK, two proteins involved in cell cycle arrest. In conclusion, DU-145 and PC-3 cells represent a useful PCa model for studying ssts trafficking/regulation, intracellular subtype-linked signaling, and validating new sst-specific SRIF analogs aimed at PCa treatment.

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Prevalence of P30L, V281L and P453S mutations of CYP21 gene in patients with nonfunctional adrenal incidentalomas

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Introduction
Clinically inapparent adrenal masses (incidentalomas) are detected incidentally by imaging procedures in 0.5–4.5% of the general population. Diagnostic process identifies functional and potentially malignant tumors. Adrenocortical tumors might be the first manifestation of non-classic congenital adrenal hyperplasia (NC-CAH). P30L, V281L and P453S mutations of CYP21 gene are the most common mutations of NC-CAH. The aim of the study was to evaluate the relationship between these mutation carriers and prevalence of adrenal incidentalomas and their clinical picture.
Description of methods/design
The total number of 100 adult patients at the age of 20–70 years with adrenal incidentalomas discovered in Computed Tomography or Magnetic Resonance Imaging were enrolled into the study after exclusion of hormonal function of the tumor. Many clinical, biochemical and imaging data were analyzed. Direct sequencing of CYP21 gene was performed to detect P30L, V281L and P453S mutations in all subjects and in the control group of 100 neonates. ACTH (Synacthen) stimulation test was carried out in all mutation carriers and among 30 randomly chosen noncarriers with adrenal incidentalomas.

Results
Eight subjects were identified to be heterozygous carriers of CYP21 gene mutations (three of P30L, three of P453S and two of V281L), whereas no mutations were detected in the neonates' control group (OR = 8.7; $P = 0.0039$). Higher prevalence of arterial hypertension (100 vs 52%), diabetes type 2 (50 vs 12%), as well as higher mean concentrations of renin, basal and stimulated 17-OHP were found in the carriers comparing to noncarriers.
Conclusion
Carrying P30L, V281L or P453S mutations of CYP21 gene is associated with higher prevalence of nonfunctional adrenal adenomas. Diabetes type 2 and hypertension are more common in selected CYP21 gene mutations carriers with adrenal incidentalomas than in noncarriers. ACTH stimulation test does not detect P30L, V281L or P453S CYP21 mutations in all carriers with adrenal incidentalomas.

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Comparison of two mitotane starting dose regimens in patients with advanced adrenocortical carcinoma

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Introduction
Current medical treatment of adrenocortical carcinoma (ACC) is based on mitotane alone or in combination with cytotoxic chemotherapy. However, very little is known about the pharmacokinetic properties of mitotane and dosing schedules are based on clinical experience only. The aim of this study was to investigate the relationship between mitotane dose and plasma concentration comparing two pre-defined treatment regimens. Secondary objectives were to evaluate safety and tolerability of mitotane treatment and to study its impact on various hormonal parameters. Time to reach mitotane plasma level of 14 mg/l was determined as a *post-hoc* endpoint.
Methods
In this prospective open-label multicenter trial, 40 mitotane-naïve patients with locally advanced or metastatic ACC were enrolled. Assignment to one of the two dosing regimens (high-dose and low-dose) was done on a case by case basis by the respective local investigator. The predefined study duration was 12 weeks. Adverse events were monitored throughout the study.
Results
Ten out of 20 patients on the high-dose regimen reached plasma concentrations ≥ 14 mg/l after a median of 46 days (18–81 days) compared to four of 12 patients on the low-dose regimen after a median of 55 days (46–74 days, $P = 0.286$). Mean cumulative mitotane dose was significantly higher in the high-dose group (440 ± 142 g vs 272 ± 121 g, $P = 0.013$). Median maximum plasma level was 14.3 mg/l (6.3–29.7) in the high-dose group ($n = 20$) and 11.3 mg/l (5.5–20.0) in the low-dose group ($n = 12$, $P = 0.235$). There was no significant difference between the two groups in the incidence and severity of adverse events.
Conclusions
The high-dose start-up regimen did not result in significantly higher mitotane levels or shorter time to reach therapeutic levels, but did result in higher cumulative doses. Toxicity in the high dose regimen was not observed to be greater than in the low-dose regimen. Hormonal changes should be expected and may need additional treatment.

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P517

The bone morphogenic proten 7 (Bmp7) plays a pro-tumorigenic role in pheochromocytoma

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Introduction
Rats carrying a germline loss-of-function mutation in p27 (MENX syndrome) develop bilateral pheochromcoytoma (PCC) with complete penetrance. Gene expression profiling of rat PCCs identified genes highly expressed in tumors vs normal adrenal medulla. Several of them were found up-regulated also in human PCCs (both sporadic and familial), including the BMP7 gene encoding a member of the bone morphogenic protein family. BMP7 has been shown to be involved in other human cancers, but its role in PCC tumorigenesis is unknown.
Methods
We used cell lines such as PC12 (rat PCC), with low endogenous levels of Bmp7, and MPC (mouse PCC) and its aggressive derivative MTT, both with high levels of Bmp7. We also use primary rat PCC cells with high levels of Bmp7. Bmp7-expressing plasmid was transfected in PC12 cells. Bmp7 gene knockdown in MPC/MTT cells and primary rat cultures was performed using lentiviral vector expressing anti-Bmp7 shRNA molecules. *In vitro* assays assessing proliferation (MTT), migration and invasion (Boyden chamber) were then performed. We analyzed the link between p27 and Bmp7 in embryonic fibroblast cells with either mutant or knocked-out p27 or in sip27- transfected PC12 cells.
Results
We observed that up-regulation of Bmp7 enhances proliferation, migration and invasion of the PC12 cells, while down-regulation of BMP7 impairs these properties in MPC and MTT cells.
Knock-down of Bmp7 in primary rat PCC cells reduced their viability compared to cells infected with control vector. Additionally, we found that p27 expression was inversely correlated to that of Bmp7, suggesting a potential functional link between the two molecules.
Conclusion
In conclusion, we observed that Bmp7 promotes the tumorigenic phenotype of PCC cells and that its level is influenced by the amount of p27. BMP7 represents a novel target for therapy of PCC since the knock-down *in vitro* shows promising impairment of the tumorigenic phenotype.

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Neuroendocrine tumours (NETs) of lung: new data on atypical carcinoid and large cell neuroendocrine carcinoma from a French-Italian multicentric study

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Introduction
Natural history of lung neuroendocrine tumours (NETs) and in particular of two histological subtypes, atypical carcinoid (AC) and large cell neuroendocrine carcinoma (LCNEC), is poorly known.
Aim of the study was to determine disease-free survival (DFS) and overall survival (OS) of sporadic, resectable, non-metastatic lung AC and LNEC.

Patients and methods
This retrospective study involved 116 consecutive patients surgically treated (R0) between 1998 and 2008 for AC and LCNEC in two Italian and French expert networks. Slides were reviewed by two expert pathologists to validate the histological definition (WHO 2004).
Results
The files of 86 patients were reviewed (49 males and 37 females). Mean age at diagnosis was 58 ± 15 years for AC and 63 ± 15 years for LCNEC. The most frequent presenting symptom was respiratory infection (AC: 24%, LCNEC: 31%); endocrine syndrome occurred in only one AC patient (Cushing's syndrome). Mean follow-up time was 80.6 months in AC and 50.6 months in LCNEC. The most used surgery was lobectomy (AC: 81%, LCNEC: 59%). N-positive status was found in 29 and 57% of AC and LCNEC respectively. The rate of recurrence was 39% among AC (15% local, 24% distant metastasis) and 41% in LCNEC (27% local, 14% distant metastasis). The mean time of recurrence from surgery was 24.6 months in AC and 15 months in LCNEC. Median OS was 5.4 years in LCNEC (not reached in AC). Median DFS was not reached in both histotypes. Recurrence rate was higher in N+ AC patients than in N0 ones (76 vs 24%).
Conclusion
Patients with lung AC and LCNEC experience a high rate of recurrence after surgery. Beside LCNEC patients, N-positive AC patients should be considered for adjuvant therapy and be subjected to an intensive monitoring on. A better definition of lung NETs prognosis and natural history is expected from large collaborative studies.

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P519

mTOR, AKT, p70S6K and ERK1/2 levels predict sensitivity to mTOR and PI3K/mTOR inhibitors in human bronchial carcinoids

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Background
Bronchial carcinoids (BCs) are rare neuroendocrine tumors that are still orphan of medical treatment. Human BC primary cultures may display resistance to everolimus, an inhibitor of the mammalian target of rapamycin (mTOR), in terms of cell viability reduction.
Aim
To assess whether the novel dual PI3K/mTOR inhibitor, NVP-BEZ235, may be effective in everolimus-resistant human BC tissues and cell lines. In addition, we search for possible markers of mTOR inhibitors efficacy, that may help in identifying the patients that may benefit from mTOR inhibitors treatment, sparing them from ineffective therapy.
Results
NVP-BEZ235 is twice as potent as everolimus in reducing cell viability and activating apoptosis in human BC tissues that display sensitivity to mTOR inhibitors, but is not effective in everolimus-resistant BC tissues and cell lines, that by-pass cyclin D1 down-regulation and escape G0/G1 blockade. Rebound AKT activation was not observed in response to treatment with either mTOR inhibitor in 'resistant' BC cells. We also show that, in addition to total mTOR levels, putative markers of BC sensitivity to mTOR inhibitors are represented by higher AKT, p70S6K and ERK1/2 protein levels.
Conclusion
These data indicate that the dual PI3K/mTOR inhibitor NVP-BEZ235 is more potent than everolimus in reducing human BC cell proliferation. 'Resistant' cells display lower levels of mTOR, AKT, p70S6K and ERK1/2, indicating that these proteins may be useful as predictive markers of resistance to mTOR and PI3K/mTOR inhibitors in human BC.

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P520

Assessment of fatal events in patients with radio-active iodine (RAI)-refractory differentiated thyroid cancer responsive to treatment with sorafenib

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Introduction
Targeted therapy with the multi-kinase inhibitor sorafenib is effective for treatment of differentiated thyroid cancer (DTC) unresponsive to RAI. Although kinase-inhibitors (KIs) are usually well tolerated, severe and even fatal adverse events are reported. Aim of the study was to assess incidence and characteristics of fatal events in patients with RAI-refractory DTC responsive to treatment with sorafenib.
Design
A retrospective analysis of patients with progressive iodine-refractory DTC subjected to off-label treatment with sorafenib in our centre was performed. Radiological response was assessed according to RECIST criteria version 1.1.
Results
From March 2010, 17 patients affected with RAI-refractory DTC were subjected to treatment with sorafenib. Of them, 12 subjects were responsive to treatment (seven achieved stable disease and five partial response). Median time of treatment for responding patients was 14 months. Fatal events were reported in five of 12 patients (42%). Three patients died from severe haemorrhage of the upper respiratory tract after 4 months of treatment. They had a wide tracheo-oesophageal neoplastic infiltration previously treated with external beam irradiation. Two subjects died from cardiac arrest after 10 months of treatment. They had developed a moderate hypertension after starting treatment with sorafenib.
Conclusions
Although treatment with sorafenib is effective in most patients affected with RAI-refractory DTC, it could be responsible of fatal events. Particularly, bleeding events and cardiac damage are considered as specific adverse events in subjects treated with KIs. In light of this, we suggest to exclude from treatment, or to use a reduced dosage, in those patients with mucosal neoplastic infiltration and those previously treated with radiotherapy. Furthermore, a careful and individualized cardiovascular management is mandatory.

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P521

Diagnosis of endogenous hyperinsulinism through arterial calcium stimulation with hepatic venous sampling

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Objective
The aim of this study was to assess the utility of arterial calcium stimulation with hepatic venous sampling (ASVS) in the localization of tumors in patients with endogenous hyperinsulinism not detected with other methods.
Patients and methods
We performed a retrospective study of 30 patients admitted to our hospital for hypoglycemia who underwent ASVS because the source of hyperinsulinism was not clearly identified by other imaging techniques. The histopathological results in patients who underwent a surgical procedure were considered the reference for statistical study of the accuracy of this technique. Statistical analysis was performed by comparing proportions with the χ^2 test with Yates' correction for contingency tables, and *Cohen's* κ coefficient as a measure of interrater agreement between two observations.
Results
Surgery was performed in 21 patients, 20 with positive ASVS and the remaining

one with negative result. Endogenous hyperinsulinism (insulinoma and nesidioblastosis) was removed in 19 patients, and all of these were detected in the ASVS. A total of 95% of the positive ASVS test were diagnosed of endogenous hyperinsulinism (80% insulinoma and 15% nesidioblastosis), and 100% of endogenous hyperinsulinism had a positive result of the ASVS being this association statistically significant ($\chi^2 = 15.771$; $P < 0.001$). A good and statistically significant agreement was obtained between histopathologic diagnostic and ASVS results. ($K = 0.518$, $P < 0.001$).

Conclusions

ASVS is a useful procedure in the localization diagnosis of endogenous hyperinsulinism not detected by other imaging tests. This technique allows tumors in the pancreatic gland to be identified and may be useful in the choice of the surgical technique to be used.

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P522

Localization of pathological parathyroids in patients with thyroid abnormalities by PTH measurements in fine needle aspiration biopsy washouts

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Preoperative localization of the pathological parathyroid gland is critical in the clinical evaluation of patients with primary hyperparathyroidism (PHP) before surgical resection. Unfortunately, scintigraphy nor ultrasound-guided FNAB do not show enough accuracy in this respect, especially in patients with concurrent thyroid pathology.

The aim of the investigation was to assess the usefulness of measurements of PTH concentration in FNAB washouts (PTH-FNAB) in diagnosing PHP in patients with thyroid nodules or chronic thyroiditis. The study included 50 patients with a focal lesion that was suggestive in ultrasonography for parathyroid gland, 40 patients with clinical and/or biochemical picture of PHP and ten patients with a suspicion for parathyroid incidentaloma. Simultaneously biopsied thyroid nodules were the internal controls.

Mean serum PTH concentration was 310.9 ± 393.9 pg/ml (mean $\pm$ SD). Positive PTH-FNAB (PTH-FNAB concentration – after dilution of a specimen in 1 ml 0.9% NaCl – higher than in serum) was observed in 40 patients (80.0%). Mean positive PTH-FNAB $\pm$ SD was 2822.41 ± 2061.29 pg/ml. Mean negative PTH-FNAB was significantly lower (12.3 ± 8.7 pg/ml, $P < 0.0002$) and similar to that in the internal controls. Negative predictive value (NPV) of classical FNAB vs PTH-FNAB was 26.5%, NPV of scintigraphy vs PTH-FNAB was 46.2%, NPV of PTH-FNAB vs scintigraphy was 66.7%. No false negative result of PTH-FNAB vs classical FNAB was noted. Lower frequency of positive PTH-FNAB was observed when the thickness of the thyroid lobes was > 20 mm (50.0 vs 87.5%, $P < 0.05$) and when the thickness of a lesion suspected for parathyroid pathology was ≤ 5 mm (66.7 vs 93.3%, $P < 0.05$). The negative influence of chronic thyroiditis was less marked (73.9 vs 85.2%). Surgical removal of the parathyroid lesion was performed in 20 patients (five cases of hyperplasia, 14 adenomas, and one parathyroid cancer). No false positive PTH-FNAB was observed in that group and only one false negative result was noted in a patient with large nodular goitre.

Measurement of PTH concentration in the FNAB washouts allows to identify pathological parathyroids in patients with thyroid abnormalities more effectively than scintigraphy and classical ultrasound-guided FNAB.

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P523

Six-years experience in the treatment of the neuroendocrine tumors with the use of peptide receptor radionuclide therapy (PRRT)

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Introduction

The aim of this study was to assess the efficacy of peptide receptor radionuclide therapy (PRRT) with the use of ^{90}Y-DOTATATE and the survival rate of patients with disseminated or non-operable neuroendocrine tumors (NETs).

Methods

In the time period from June 2006 to October 2012, 70 patients were treated with PRRT in our department. The ^{90}Y-DOTATATE therapeutic activity was calculated per total body surface area up to a total of 7.4 GBq/m^2 administrated in three to five cycles, repeated every 4–9 weeks. Before and after the therapy, blood tests for hematology, kidney and liver function were performed. After PRRT, patients have been further treated with cold long acting somatostatin analogues (Sandostatin LAR) to the progression of the disease.

Results

Out of 70 ^{90}Y-DOTATATE treated patients, 22 died after completing the therapy, among them two due to myocardial infarction. After 12-month follow-up, stabilization of the disease was observed in 63%, partial remission in 25%, and progression in 12% in this group of patients. The progression-free survival (PFS) was found to be 41.27 months and the event-free survival (EFS) 37.73 months. The median overall survival (OS) was not reached. During follow-up, transient decrease in PLT, WBC and hemoglobin values was observed. A increase in creatinine level and decrease in GFR values over observation period were found, but these were clinically insignificant symptoms of transient nephrotoxicity.

Summary

Long-term patients benefit in the form of long survival rate, symptomatic relief and tumor mass reduction after ^{90}Y-DOTATATE therapy was observed. PRRT is safety method and may extend the survival of disseminated patients with NETs.

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P524

The expression of SSTR dominant negative truncated variant sst5tMD4 influences the effects of SOM-230 on prostate cells *in vitro*

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Introduction
The presence of truncated variant of somatostatin receptors (SSTR) has been demonstrated in pituitary tumours. These variants seems act as dominant negative of SSTR and induce the resistance to SST analogue (SSTA) treatment. A variant of SSTR5, sst5TMD4, has been found to disrupt SST signalling in breast cancer cells. In present study, we assayed the expression of sst5TMD4 in two non-transformed epithelial prostate cell lines (EPC): EPN, derived from a normal surrounding area of a prostate tumour, and CPEC, derived from the core of a prostate cancer tissue. In these cells, we evaluated the effects of SOM-230 (Novartis, Basilea, SW), a SSTA pan ligand with strong affinity for SSTR2 and 5. Cell cultures starved in red phenol-free DMEM and 1% charcoal treated FBS for 5 d were treated either with 10-6 SOM-230 or 10-8 SOM-230. After 24/48 h, cells were harvested for RT-PCR and for SDS–PAGE/western blot, or labelled with presidium iodide for cell cycle analysis by flow cytometer.
Results
SSTR2 and 5 were equally expressed in both cell lines. SSTR1 and 3, and sst5TMD4 mRNA levels were higher in CEPC than in EPN ($P<0.001$). In EPN, SOM 10-6 induced a significant caspase-dependent apoptosis, a reduction of S-phase proliferation together with an increase in bcl2 and a decrease in c-myc expression. In CPEC cells, SOM-230 treatment resulted in a modest apoptosis induction and a slight inhibition of cell growth, without changes of bcl-2 and c-myc levels.
Conclusions
sst5TMD4 variant is differently expressed in the EPC lines studied here. SOM-230 is effective in the control of cell growth in EPN cultures, while the reduced apoptotic response and the lack of growth arrest observed in CEPC could be due to presence of high levels of sst5TMD4 interfering with SST signalling.

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P525

Function and origin of tumour-associated fibroblasts (TAFs) in human parathyroid neoplasia

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Tumour-associated fibroblasts (TAFs) are important players in tumour formation, growth, enhancement and metastasis. We firstly investigated the TAFs component in human parathyroid neoplasia from patients with primary hyperparathyroidism. Alpha-smooth muscle actin (alpha-SMA) has been used to identify activated TAFs (myofibroblasts). Culturing explants from parathyroid adenomas (PA, $n=5$), large spindle-shaped alpha-SMA+ cells outgrew from explants and the expression of activated fibroblasts markers such as vimentin, stromal derived factor-1 (SDF-1/CXCL12) and fibroblast activated protein were detected. Immunohistochemistry showed alpha-SMA+ cells highly represented in normal parathyroid glands ($n=3$) where they lined the acinar structures. In typical PAs ($n=5$) alpha-SMA+ cells were variably reduced, though they surrounded new microvessels suggesting a role in neoangiogenesis. Interestingly, in human fetal parathyroids (19–24 weeks of gestation), myofibroblasts were exclusively found lining blood vessels. In atypical adenomas ($n=3$) and carcinomas ($n=3$), the chief cells proliferating in sheets were not sustained by myofibroblasts, which were highly represented in the fibrous bands and capsula stroma, suggesting a role of alpha-SMA+ cells in invasiveness. Coculture of human bone-marrow mesenchymal stem cells (hBM-MSCs) with PA-derived explants ($n=3$) induced significant increases of *VEGFa* mRNA expression levels in hBM-MSCs. Immunofluorescence (IF) and FACS ($n=5$) identified 32–63% of PA-derived cells expressing CXCR4, the SDF-1 receptor, whose 47–90% coexpressing PTH and CXCR4. Treatment of cocultures with the CXCR4 antagonist AMD3100 reduced the coculture-stimulated *VEGFa* mRNA expression in hBM-MSCs, suggesting that the proangiogenic effect might be regulated through the CXCR4/SDF-1 pathway. A subset of alpha-SMA+ cells were shown by IF to co-express the haematopoietic marker CD34 suggesting they might be perivascular adipose-tissue derived mesenchymal progenitors. A subset of alpha-SMA+ cells also co-expressed the parathyroid marker GCM2, and the endodermic transcription factor TBX1, suggesting they might derived from chief cells through epithelial-to-mesenchymal transition. In conclusions, we identified in parathyroid neoplasia cells showing features of activated TAFs that might be involved in tumoral neoangiogenesis and invasiveness.

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P526

Expression of FGF23, Klotho, CaSR, and PTHrP in carcinoma *in situ* and germ cell tumors of the testis: implications for testicular microlithiasis

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Introduction
Vitamin D (VD) metabolism is active in normal testis, but lost during the malignant progression from carcinoma *in situ* (CIS) to overt germ cell tumors (TGCTs). CIS and TGCTs are often associated with intratubular deposition of hydroxyapatite (microlithiasis). Imbalance in FGF23 and PTH homeostasis may result in calcification of mesodermal-derived tissue, so we hypothesized that this mechanism may be involved in testicular microlithiasis. Here, we investigated classical regulators of VD metabolism, phosphate and calcium homeostasis: FGF23, CaSR, PTHrP, in addition to their corresponding receptors and downstream mediators in human testis, with and without CIS and TGCT.
Materials and methods
In total, 20 samples of normal adult human testis, 20 CIS, 25 TGCTs and five fetal testes were investigated by qPCR and immunohistochemistry. FGF-23 was measured with an immunoassay in serum and seminal fluid of ten patients with TGCT.
Results
Normal germ cells expressed PTHrP, PTHR, Klotho, FGFR1, FGFR3 and the phosphate transporter NPT2b, but virtually no FGF23 expression was detected. FGF23, CaSR, PTHrP and NPT2a were markedly upregulated ($P<0.05$) in CIS at RNA and protein level, with a high correlation between CaSR and PTHrP ($r=0.91$ $P<0.005$) and a correlation of PTHrP and FGF23 with NPT2a ($P<0.01$). PTHrP, but not FGF23, was expressed in fetal germ cells. The protein expression of FGF23, CaSR, and PTHrP diminished in invasive TGCTs, and FGF23 levels in serum and seminal plasma were not significantly higher in TGCTs patients compared with controls.
Conclusion
Testicular neoplasia is associated with profound changes in intratesticular VD, calcium and phosphate homeostasis. It remains to be established whether over-expression of FGF23 in CIS is a regulatory mechanism, or reflects genomic amplification associated with malignant transformation, but upregulation of FGF23 may contribute to the formation of testicular microliths in the vicinity of CIS and TGCTs.

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P527

The clinical characteristics of primary hyperparathyroidism (PHPT) in patients with multiple endocrine neoplasia (MEN) type 1

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Rarely PHPT could be a part of MEN 1 (2–4.5%). Little is known about clinical differences between MEN1 related and sporadic PHPT.
Aim
To compare the clinical features of PHPT in MEN type 1 cases and sporadic PHPT.
Materials and methods
Data were obtained in 442 patients: 62 sharing MEN1 phenotype (PHPT in association with pituitary adenomas and/or gastroenteropancreatic neuroendocrine tumors) (Group-I) and 380 with apparently sporadic PHPT (Group-II). Serum Ca, intact PTH, urine Ca, 25-OH-VitD, osteocalcin, β-CTx) and BMD *z*-score measurements, renal ultrasonography, visualization of parathyroid glands were performed in both groups. The additional diagnostic tests (prolactin levels, ultrasound of pancreas and adrenals) were performed in patients to exclude or evaluate other than PHPT endocrine disorders.
Results
The age at diagnosis was younger in Group I (39.5 (28–51) years) than in Group II (57 (48–64) years), $P<0.001$. In 50% of Group I PHPT debuted before age of 40 years, whereas in Group II in 70% of patients the diagnosis was made after age of 50 years. The distribution of males/females was 1:2.6 in Group I and 2:10 in Group II. In 93% of Group II PHPT was caused by a single parathyroid adenoma, in Group I in 59.6% an enlargement of multiple parathyroid glands was observed. In 64% of iPTH was higher than upper normal range less than 2.5 times, whereas in Group II 54% was more than three times higher than normal limits. In the meantime the levels of Ca in both groups were not significantly different ($P=0.28$). The mild PHPT was observed in Group I more frequently than in Group II (33.8 vs 19%). PHPT presented with osteoporosis with the same prevalence in both groups, but with more frequent renal manifestations in Group II. In Group I the decrease in BMD at all sites and nephrolithiasis developed independently of the degree of PTH elevation, as opposed to Group II. Multifactorial analysis revealed the combination of moderately elevated PTH and the age at diagnosis younger than 40 years to increase the risk of MEN1-related PHPT in these patients independently of their gender and the mild course of PHPT.
Conclusion
The patients with MEN1-related PHPT are younger at the diagnosis of PHPT, have more frequently multiple enlarged parathyroid glands and show mostly mild course of disease (especially in young age) and less increased PTH compared with those with sporadic PHPT.

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Establishment of patient-individual tumor models for endocrine tumors

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In an attempt to amend the lack of preclinical models for endocrine tumors, we recently initiated establishment of patient-individual tumor models. Pieces from four adrenocortical carcinomas (ACC), one aldosterone producing adenoma (APA), one pheochromocytoma (pheo), one metastasis of a malignant pheo, one pheo and medullary thyroid carcinoma associated with multiple endocrine neoplasia type 2 and 11 neuroendocrine tumors (NETs, nine NETs of the gastroenteropancreatic system including three metastasis as well as one primary tumor and one metastasis of Merkel cell carcinoma) were implanted subcutaneously into immunodeficient mice. CD31 analyses after tumor explantation revealed heterogeneous grades of vascularization for ACCs and NETs (from high to absent) which seemed to be overall not dependent on tumor origin. Single explants (E) showed often ki67 indices (%) and SF-1 (positive cells/six high power fields) or chromogranin A stainings (ChrA morphology and stained area as % of total) comparable ($P>0.05$) to the original patient tumor (ACC1 ki67, patient: 7.9 ± 2.3, E: 8.9 ± 2; SF-1, patient: 4.2 ± 0.7, E: 4.2 ± 0.6; NET1 ki67, patient: 7.3 ± 1.4, E: 7.7 ± 1.1; ChrA, patient and E: 30–60%, nest-shaped). However, various explants revealed intra-tumoral heterogeneities maybe reflecting different regions within one patient's tumor (ACC2 ki67, patient: 9.7 ± 2.6 vs E1: 2.8 ± 0.9, $P<0.05$; E2: 2 ± 1.2, $P<0.05$; E3: 11.3 ± 3.3, $P>0.05$; SF-1, patient: 3.3 ± 0.4 vs E1: 3.2 ± 0.4, $P>0.05$; E2: 0.5 ± 0.1, $P<0.0001$; E3: 1.1 ± 0.2, $P<0.0001$; NET2 ki67, patient: 2 ± 1 vs E1: 10.3 ± 0.7, $P<0.0001$; E2: 7.1 ± 0.9, $P<0.001$; E3: 13.1 ± 1.9, $P<0.0001$; E4: 2.7 ± 0.6, $P>0.05$; ChrA, patient: nest-shaped 60 ± 7.1, E1: nest-shaped 21.9 ± 3.5; E2: 8.8 ± 7, nest-shaped; E3: 0 ± 4.2, diffuse; E4: 10 ± 3, diffuse). APA and pheos were low or not vascularized and ki67 indices were comparable with patient tumors (pheo, patient: 2.9 ± 0.4, E1: 2.9 ± 0.7, E2: 2.5 ± 0.6; APA, patient: 3.2 ± 0.9, E1:1.6 ± 0.5, E2: 3.6 ± 1.7). During ongoing preclinical studies we plan to include groups with patient-tumor bearing mice to investigate putative applicability in therapeutic settings.

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Familial malignant paraganglioma is long-term stabilized with the tyrosine-kinase inhibitor sunitinib

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Introduction
Paragangliomas are neuroectodermal tumors that arise from adrenal medulla or extra-adrenal ganglia and are characterized by high vascularisation. A high rate of these tumours is genetically inherited. For malignant paragangliomas, chemo- and radio-therapy are potentially effective, but tumor response is of short duration and patient prognosis is quite poor. Sunitinib is a tyrosine-kinase inhibitor, targeting VEGFR1, -2, PDGFRα, -β, RET and c-Kit. Recent experiences highlighted that sunitinib is potentially effective in patients with malignant paraganglioma. Our data aim to suggest a long-term use of sunitinib to arrest progression in familial paragangliomas.
Case report
Two patients (female, 48 years; male, 27 years) affected with persistent post-surgical malignant abdominal paraganglioma associated with paragangliomatosis type 4 (*SDHB* mutations) were treated with sunitinib 37.5 mg/day. One of them experienced a partial response and one other a tumour stabilization. At the last evaluation, 42 months after starting sunitinib, biochemical parameters (24-hour urine catecholamines and metanephrines) were in the normality range and tumor lesions were stable. Sunitinib was well tolerated and no serious adverse event was observed in both patients.
Conclusions
Sunitinib seems to be an effective agent for the management of patients with unresectable/advanced familial malignant paragangliomas. The striking finding of this report is that sunitinib at the dose of 37.5 mg/day is able to obtain long-time stabilization of tumor progression.

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Insulinoma: is enucleation a safe option?

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Background
Insulinomas are the most prevalent functional neuroendocrine tumors of the pancreas. Enucleation is often preferred to pancreatic resection because it's minimally invasive nature. The aim of this study is to assess the post-operative surgical outcome in particular pancreatic fistula after resection.
Methods
All patients with insulinomas were selected from a retrospective database of resected pancreatic neuroendocrine tumors (pNET). Patients were operated between 1992 and 2012. Surgical procedure and post-operative outcome were analysed.
Results
A total of 109 patients with pNET underwent resection, 22 patients had an insulinomas (20%), 12 women and ten men with a median age of 50 years (21–83 years). Three patients had MEN-1 syndrome (14%). Somatostatin receptor scintigraphy was performed in 12/22 patients and showed accumulation of the radiopharmacon in the insulinomas in two patients. Twelve patients underwent enucleation of the insulinoma, $n=8$ located in the head, $n=2$ in the tail, $n=2$ in the central/tail region. All enucleated insulinomas were smaller than 20 mm. Ten patients underwent pancreatic resection, $n=6$ tail resection, $n=3$ corpus/tail resection, $n=1$ head/corpus resection including pancreatic jejunostomy.

Complications occurred in 7/12 (58%) enucleation patients and in 4/10 (40%) patients with a pancreatic resection ($P=0.4$). The most frequent complication was pancreatic leakage in 9/22 (41%), respectively 7/12 for enucleation and 2/10 for pancreatic resection ($P=0.1$). Two patients developed diabetes mellitus post-operatively; both patients were diagnosed with recurrent insulinoma and underwent multiple pancreatic resections. One MEN-1 patient developed metastatic disease of the insulinomas after resection of the primary.
Conclusions
Enucleation can be frequently performed in 55% for insulinomas and it's a less invasive operation technique without endocrine insufficiency but with a high complication rate, especially for pancreatic fistula. Although insulinomas are usually small, a pancreatic resection is a safe alternative option. Careful patient selection and operation should be performed.

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Long-term outcome after transarterial chemoembolization of hepatic metastases from neuroendocrine tumors

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Introduction
Most patients with neuroendocrine tumors (NETs) present with liver involvement at the time of diagnosis. Trans-catheter arterial chemoembolization (TACE) is accepted treatment of patients with non-resectable hepatic metastases from NETs.
Aim
To analyze objective tumor response and clinical outcome in patients with hepatic metastases from NETs who underwent TACE.
Methods
Thirty-one patients underwent 140 TACE procedures. Kaplan–Meier method was used to calculate the progression-free survival (PFS) and overall survival (OS). Tumor responses were measured by CT and MRI, and were assessed using the RECIST criteria.
Results
NET originated from the pancreas ($n=5$), small bowel ($n=11$), colon ($n=1$), lung ($n=5$), unknown primary localization ($n=9$). Almost all patients (93.5%) had received medical treatment including octreotide, interferon-α, chemotherapy, and peptide-receptor-radionuclide therapy. According to WHO criteria, majority were well-differentiated neuroendocrine carcinomas (71.4%). Median OS for all NETs was 59 months (4–180) with 5-year survival of 58.9%. The 5-year OS rates for patients with pancreatic, carcinoid and unknown primary tumors were 33.3, 75 and 38.9% respectively. Median PFS for all patients was 32 months (1–139). The only group of patients with 5-year PFS was the one with carcinoid tumor (59.3%). None of the 31 patients had a complete response. After third TACE a partial response was observed in two, stable disease in eight patients and there was none of the patients with the progressive disease. After the fifth TACE partial response was observed in three, stable disease in five and progressive disease in four patients.
Conclusion
The OS after TACE for all NETs is approximately 5 years, and median time to progression is about 32 month. Patients with carcinoid tumors had better outcomes than others. TACE is effective in stabilization and reduction of tumor growth in patients with hepatic metastasis from NETs.

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Pituitary transforming gene 1 (PTTG1) expression in seminoma

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Pituitary transforming gene 1 (PTTG1) is a mammalian securing involved in mitosis to ensure chromosomal stability. PTTG1 is overexpressed in a variety of tumors. It can directly and indirectly induce expression of genes that are involved in regulating tumorigenesis and cancer development (c-Myc, bFGF, VEGF and MMP2).
We evaluated PTTG1-expression by immunohistochemistry on formalin-fixed and paraffin-embedded specimen testicular tissues from 53 male patient underwent to therapeutic orchidectomy, collected from 2006 to 2011.
PTTG1 staining was located in the nuclear and cytoplasm of neoplastic cells. Within the tumor we identify a peripheral zone as edge of neoplasia and neoplastic tissue up to 1 mm towards interior of the tumor and central zone as neoplastic areas further than 1 mm from the border of the tumor. Different staining in subcellular localization (nuclear and cytoplasm) was observed in peripheric area vs central area of neoplastic lesion. In the peripheric area, PTTG1 immunoreactivity was detected mostly localized in the nucleus, while in the central area PTTG1 staining was evident more intensely in cytoplasm of positive elements.
PTTG1 expression was significantly lower in the central when compared with the peripheral area, with a greater number of positive cells in the borders of the tumor and gradient periphery/center significantly correlate with the size of the tumor.
PTTG1 positive staining was also reported in the peritumoral region a nuclear staining prevalent pattern.
We firstly described neoplastic PTTG-1 positive cells with nuclear and cytoplasmatic staining in seminoma. Our distribution data support the idea that PTTG1-expression in neoplastic cells in the front of the tumor infiltration and in the intertubular areas might respond to demand of the tumor cells to move and invade surrounding tissue, increasing tumor angiogenesis. Further investigation are required to clarify if a functional abrogation of PTTG-1 could provide new therapeutic approaches in the management of seminoma.

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Polymorphisms of the glucocorticoid receptor gene, as phenotype modifiers in patients with hormonally inactive adrenal adenomas

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Introduction
Altered sensitivity against glucocorticoids is partly influenced by polymorphisms (SNP) of the glucocorticoid receptor gene (GR). The aim of the present study was to extend our earlier study by inclusion and evaluation the role of the BclI and A3669G polymorphisms of the GR in patients with hormonally inactive adrenal adenomas.
Description of methods
This is a retrospective, single-centre genetic association study The study included 99 patients with hormonally inactive (HI) adrenal adenomas discovered incidentally (incidentalomas) and 129 healthy controls. Hormonal evaluation of the hypothalamo–pituitary–adrenal axis, measurement of metabolic parameters was carried out in patients, and genetic analysis in all subjects. The BclI polymorphism was detected by allele specific PCR while the A3669G by Taqman allele discrimination assay.
Results
The prevalence of the BclI was lower in unilateral while the prevalence of the A3669G was lower in patients with bilateral HI than in healthy controls, (BclI: 21.8 vs 34.5%; A3669G: 10.8 vs 22.1% $P<0.05$). Patients who carried the BclI polymorphism had higher: systolic blood pressure, BMI, serum cholesterol level and ACTH level measured after methopyron test. The prevalence of obesity was also higher in BclI carriers, and this prevalence further increased when the A3669G SNP was present. Although lower prevalence of hyperlipidemia and plasma ACTH level was detected in the same group.
Conclusion
Our results confirm that behind the development of adrenal tumors especial in bilateral cases the polymorphic allele of the N363S and the wild type alleles of the BclI and A3669G represent genetic risk factors. Different mechanisms related to GR genetic variants alone or together may contribute to the morbidities found in these patients

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Mitochondrial ultrastructure in pseudohypoxic succinate dehydrogenase B and von Hippel–Lindau gene mutation derived pheochromocytomas and paragangliomas

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Mutations in the mitochondrial succinate dehydrogenase (SDH) subunits A, B, C, and D have been shown to hamper oxidative phosphorylation and predispose to pheochromocytomas (PHEOs) and paragangliomas (PGLs). These tumors are characterized by a glycolytic and pseudohypoxic phenotype, which is also seen in most PHEOs/PGLs occurring as part of von Hippel–Lindau (VHL) syndrome, due to VHL gene mutations. The rate of extra-adrenal tumor origin and malignancy however is particularly high in SDHB-PHEOs/PGLs, while VHL-PHEOs/PGLs are almost always adrenal and benign.

We have recently shown that despite decreased complex II activity, mRNA and protein abundance of several oxidative phosphorylation subunits is elevated in SDHB compared to VHL PHEOs/PGLs.

Here we present ultrastructural evidence for vast differences in number and appearance of mitochondria in SDHB and VHL derived PHEOs/PGLs, as we have previously presented on a smaller patient cohort. Forty-three tissue samples of SDHB (47% metastatic) and VHL patients (0% metastatic) have been evaluated. In SDHB PHEOs/PGLs density and size of mitochondria was increased compared to VHL PHEOs/PGLs with a disrupted cristae structure in both types of PHEOs/PGLs. Western blot for the mitochondrial marker cytochrome C revealed higher levels in SDHB PHEOs/PGLs. No difference was observed between metastatic and non-metastatic SDHB tumors.

Our data indicates that disruption of the respiratory chain caused by SDHB mutations may lead to an accumulation of abnormal, possibly dysfunctional mitochondria. Further studies on live cells are mandated to conclude if the disrupted appearance of the mitochondria leads to a loss of mitochondrial membrane potential and functionality or reactive oxygen species generation and the potential role of aberrant mitochondria in metastatic potential.

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Adrenal lesions in patients with neuroendocrine tumors

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Introduction

The coexistence of adrenal tumors (AT) in patients with neuroendocrine tumors (NETs) has not been extensively studied. The aim of our study was to investigate their prevalence and clinical significance in these patients.

Materials and methods

We retrospectively studied 447 patients with NETs of all localizations, treated at our department between 2004 and 2012. Diagnosis was established pathohistologically, classification performed according to stage and grade as proposed by ENETS, and disease progression monitored by CT/MRI imaging. The nature of AT was evaluated pathohistologically when possible, or by CT/MRI characteristics indicative for benign/malignant lesion. Functional status was assessed appropriately in all patients. Statistical analysis was performed by SPSS software.

Results

The prevalence of ATs was 14.3% (64 patients). The involvement was unilateral in 59.4% (42.2% left and 15.6% right adrenal) and bilateral in 40.6% of patients, with mean diameter of 30.2 ± 20.9 mm (9–108 mm). The majority of patients had primary pancreatic NET (40.6%), followed by lung NET (28.1%), NET of unknown primary (9.4%) and intestinal NET (7.9%), while other localizations were present with less than 4.7% each. Ten patients (15.6%) had MEN1 syndrome. Metastatic nature was pathohistologically verified or was indicative by CT/MRI characteristics in majority of cases (55.6%, 35 patients), five of which (14.3%) had MEN1. Out of the remaining 29 patients with benign lesions (6.5% of all patients with NETs), size progression was noted in only one AT, and only one tumor was functionally active (subclinical Cushing's syndrome). Adrenal involvement positively correlated with tumor grade ($r=0.389$, $P<0.001$) and stage ($r=0.261$, $P=0.007$). Overall survival of patients with coexistent AT was 77.0 months (95% CI 23.9–130.1), which was not significantly different than OS of patients without AT ($P>0.05$).

Conclusion

Adrenal tumors in our group of patients with NETs were mostly metastatic, but their overall clinical relevance needs further studies.

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Association between expression of Ki-67, parafibromin, p27, and Rb protein and the biological behavior in parathyroid tumors

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Background

Parathyroid carcinoma (PC) is uncommon and occurs in only 1–5% cases of primary hyperparathyroidism. The histological diagnosis of parathyroid tumors can be challenging, and beneficial histological markers are not available for diagnosis or predicting the prognosis of patients. In this study, we investigated the expression of Ki-67, parafibromin, p27, and Rb protein in parathyroid tumors and assessed the correlation between histological diagnosis or prognosis, by using immunohistochemical staining in patients with confirmed PC and parathyroid adenoma.

Methods

Thirty-eight formalin-fixed, paraffin-embedded tissue samples of surgically resected PC ($n=18$) and parathyroid adenoma ($n=20$) were studied by immunohistochemical analysis for Ki-67, parafibromin, p27, and Rb expression.

Results

Positive findings were noted for Ki-67 in 11 (61.1%) cases of carcinoma and none of the cases of adenoma ($P=0.0002$), for parafibromin in one (5.6%) case of carcinoma and 19 (95%) cases of adenoma ($P< 0.0001$), for p27 in three (16.7%) cases of carcinoma and all cases of adenoma ($P< 0.0001$), and for Rb in one (5.6%) case of carcinoma and 13 (65.0%) cases of adenoma ($P<0.0001$). Furthermore, the high rate of Ki-67 expression and the weak immunoreactive expression of parafibromin, p27, and Rb may indicate poor prognosis of PC. These results indicate that the use of Ki-67, parafibromin, p27, and Rb may be helpful in the assessment of parathyroid tumors. Furthermore, the high rate of Ki-67 expression and the weak immunoreactive expression of parafibromin, p27, and Rb may indicate poor prognosis of PC.

Conclusions

These results suggest that Ki-67, parafibromin, p27, and Rb expression may be helpful markers for diagnosis and predicting the clinical outcome of patients with PC.

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***RPS13* and cell cycle signaling pathways in pituitary tumorigenesis**

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Introduction

Abnormalities in cell cycle pathways, such as CDKN1B (p27) underexpression, have been identified in the pathogenesis of pituitary adenomas, but their underlying mechanisms have not been elucidated. Ribosomal proteins have been recently related to pituitary tumorigenesis. In gastric cancer, RPS13 down-regulates p27 and promotes cell cycle progression; these mechanisms have not yet been explored in pituitary adenomas.

Objective
To evaluate the relationship between *RPS13* and *CDKN1B, CDK2*, *CCNE1, MYC* gene expression in pituitary tumorigenesis and its association to clinical findings.
Methods
We studied four groups: non-functioning pituitary adenomas (NFPA, $n=21$), GH-secreting adenomas ($n=18$), ACTH-secreting adenomas ($n=12$) and normal pituitaries ($n=07$). Clinical and pathological data were collected. RNA was isolated by TRIzol method. Gene expression was assessed by qRT-PCR. Kruskal–Wallis test was used for continuous variables between groups and Fisher Exact test for categorical data.
Results
Differential gene expression among the groups were observed in *CDKN1B* ($P=0.03$), *CCNE1* ($P=0.02$) and *MYC* ($P=0.002$), but not *RPS13* ($P=0.1$) and *CDK2* ($P=0.07$). We observed *CDKN1B* underexpression in somatotrophinomas, *CCNE1* overexpression in NFPA and *MYC* underexpression in NFPA. In corticotrophinomas, we found no association between gene expression and tumor size, remission or immunohistochemistry (IHC). In somatotrophinomas, no relationship was found between gene expression and tumor size, visual field, IGF-1 levels, basal and post-oGTT GH levels, IHC, post-surgery remission and disease control. Tumors with higher *CDKN1B* expression tended to achieve control with somatostatin agonist ($P=0.08$). In NFPA, higher *CDK2* expression was associated to *null cell* subtype ($P=0.03$) with a tendency to correlate with tumor size ($P=0.08$). Higher *CCNE1* expression was associated with remission ($P=0.02$).
Conclusion
The p27-CDK2-CCNE1 pathway seems dysregulated in pituitary adenomas and may interact with other aberrant pathways, leading to an environment that may have putative role in pituitary tumorigenesis. Overexpression of *RPS13*, however, does not seem to be the underlying mechanism.

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Incidental thyroid carcinomas in patients operated on for benign thyroid pathology, are there preoperative factors suggesting a higher risk?

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Introduction
The discovery of incidental thyroid carcinomas (ITC) in patients undergoing surgery for a benign disease range between 3 and 16% of cases. During years, patients with hyperthyroidism were considered protected by TSH suppression (TSH is well known factor that favors the development of thyroid cancer). Thyroid size might be a predictive factor due to the excess of thyroid tissue prone to mutations. The knowledge of determining factors foretelling the presence of undiagnosed thyroid carcinomas would be interesting since these tumours not always have an indolent course.
Description of methods/design
We evaluate retrospectively 568 patients with total thyroidectomy operated on for reasons other than malignancy between 2005 and 2011. We compare demographic and clinical characteristics between two groups: patients without ITC and patients with discovery of ITC in final pathological study of thyroid. Analysis included age, sex distribution, preoperative hyperfunction, pathology of benign tissue (adenoma vs hyperplasia/inflammation), and thyroid size measured by weight.
Results
ITC was discovered in 79 of 568 operated patients (13.9%). Average age in patients with ITC was 54.6 ± 14.6 years while 55.1 ± 12.1 years in non-ITC patients ($P=0.75$). Mean weight in thyroid harboring ITC (54.2 ± 43 g) was significant lesser than in non malignant glands (76.9 ± 76.2 g; $P=0.011$). There were 84 hyperthyroid patients, ten in the ITC group, without significant difference ($P=0,73$). Final diagnosis included 152 adenomas and 416 hyperplasic or inflammatory glands, without difference between both groups (χ^2 $P=0.79$). Sex did not influence in the detection of ITC, with nine cases in 69 males, and 70 in 499 females.
Conclusion
The incidence of ITC in our thyroidectomized patients is 13.9%, similar to previously published. Neither age nor sex were useful clues to suspect ITC. Thyroid hyperfunction does not protect from ITC. Adjacent pathology is not relevant to the appearance of ITC in an operated benign thyroid. The only parameter associated with more prevalent ITC was a lesser weight of the dissected thyroid.

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Involvement of PKCβ and PKCδ isoforms in TSH signaling pathway in thyroid cancer cell lines

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It is well established that most TSH effects on the thyroid gland, including stimulation of proliferation, thyroid hormone synthesis and expression of thyroid-specific genes, are transmitted mainly by the adenylate cyclise pathway. However, in human follicular cells and in rat FRTL-5 cells, TSH can also stimulate the β-isoforms of PLC that catalyzes the hydrolysis of phosphatidyl-inositol 4,5-phosphate, yielding the second messengers DAG and inositol 1,4,5-phosphate facilitating an increase in intracellular Ca^{2+}. In FRTL-5 cells TSH has been suggested to increase DAG via phospholipase D, which produces DAG from phosphatidylcholine hydrolysis, suggesting an alternative mechanism for TSH-dependent activation through protein kinase C (PKC).
In the present study, we characterize the PKCβ and PKCδ isoforms expression and function in human follicular carcinoma cells, FTC133, and in human transformed thyrocytes, Nthy-ori cells, in order to understand whether these PKC isoforms are involved in TSH-mediated follicular cell proliferation and apoptosis. We mainly focus on PKCβ and PKCδ isoenzymes which are the most abundantly expressed isoforms in several tissues, are the most extensively studied and have two opposing roles in regulating cell proliferation.
In the Nthy-ori cells TSH stimulated cell proliferation and protected from apoptosis with a PKC-mediated mechanism. At the contrary, TSH did not increase FTC-133 cell viability nor protected the cells from PKC-inhibitor induced apoptosis. However, in FTC-133 cells TSH induced PKC expression, as well as downstream PKC targets GSK3β and AKT phosphorylation through a PKC-mediated mechanism. Moreover, immunofluorescence showed PKCβ and PKCδ perinuclear and citosolic location. These data suggest that TSH plays different roles in normal vs neoplastic thyrocytes.
Further studies are needed to clarify the role of PKCβ and PKCδ in the TSH signaling pathway in thyroid cells.

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P540

PKCδ plays an important in regulating human medullary thyroid carcinoma cell viability

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Protein kinase C (PKC) is a family of serine-threonine kinases that regulate many cellular processes including proliferation and survival. Previous evidence has shown that PKC is involved in the control of human medullary thyroid carcinoma (MTC) proliferation and survival by modulating apoptosis, with a mechanism that implicates PKCβII isoform and translocation in different subcellular compartments.
In this study, we investigated the role of PKCδ signaling in the proliferation of a human MTC cell line, the TT cells. We found that pharmacological inhibition of the PKCδ pathway with Rottlerin reduces caspase 3/7 activity. Using a shRNA vector system, which provides more than 90% gene expression inhibition, we found that cell proliferation is greater in PKCδ-defective-TT cells than in mock-transfected cells, this difference being significant after 3 days. In addition, we found that PKCδ silencing reduces STAT5(Y694-699) phosphorylation but not AKT(Ser473) and p70S6K(T389) phosphorylation, all downstream targets of PKC pathway involved in cell growth, cell cycle and proliferation.
Moreover, we demonstrated that PKCδ silencing increased human VEGF secretion after 4 days.
These observations indicate for the first time that PKCδ pathway plays an important role in the growth control and VEGF secretion of human MTC cells.
Key words
PKCδ, cell viability, TT cell lines

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P541

Sequential use of the kinase-inhibitors sorafenib and sunitinib in a patient affected with pluri-metastatic iodine-refractory follicular thyroid carcinoma

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Introduction
Kinase-inhibitors (KIs) are effective for treatment of most aggressive endocrine cancers. The crucial point about treatment with KIs is that these agents are not curative and their effects are at best transitory and are always followed by a restoration of tumour growth and progression. Several retrospective and phase II studies demonstrated efficacy of both sorafenib and sunitib for treatment of iodine refractory differentiated thyroid cancer although results from phase III trials are not available yet.
Case report
A 57 year old woman was affected with a pluri-metastatic iodine refractory follicular thyroid carcinoma. She presented multiple metastases in lymph nodes (both cervical and mediastinal), lung, liver, bone. Treatment with sorafenib was started and a radiological response was achieved (stable disease according to RECIST criteria). Nevertheless, effectiveness of sorafenib was not durable: progression of disease was restored after 7 months of treatment. Then treatment with sunitinib was started. Interestingly, sunitinib obtained a clear morphological regression of disease (partial response according to RECIST). At present treatment with sunitinib (lasting for 11 months) is still ongoing and disease is still not progressive.
Conclusions
KIs may lead to arrest of tumour progression and improvement of survival. However, disease is expected to escape during therapy. For this reason, to identify a therapeutic scheme based on a sequential use of different KIs could delay the development of tumor progression.

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P542

Endogenous Cushing's syndrome: the Filipino clinical experience of 19 cases

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Background and significance
Endogenous Cushing's syndrome is a rare disease entity approximately 13 cases per million population. In Asia, and specifically in the Philippines incidence is unknown. As well, it's clinical presentation among Filipinos is not well described. The local epidemiology of obesity, diabetes and hypertension are different from other countries. These conditions are the usual initial differential diagnosis for Cushing's syndrome. This report on a collection is thus important for the awareness of clinicians on the clinical presentation and course of individuals with this condition.
Methodology
Medical records of patients diagnosed with Cushing's syndrome within the year 2005–2011 were retrieved using the ICD-10 code for Cushing's syndrome (E24). All selected medical records were individually reviewed, assessed and carefully recorded.
Results
The study included 19 cases (eight pituitary adenoma, seven adrenal adenoma, four ectopic adenoma) of endogenous Cushing's syndrome. Female predominance was noted. The most commonly reported clinical symptom and sign were weight gain and moon facies respectively. Short clinical course, hyperpigmentation, ecchymoses, behavioural changes and severe hypokalemia were observed to be more prominent among cases with ectopic sources. All cases had significantly elevated midnight serum cortisol level and 24 h urine cortisol with ectopic sources exhibiting the highest level. There was no observed correlation between the tumour size and level of serum cortisol in all types. Majority underwent tumour resection leading to complete reversal of steroid excess.
Conclusion
Adrenal, pituitary and ectopic foci are the main sources of endogenous steroid excess. Ectopic ACTH syndrome manifests differently with other causes providing clues that might help in rapid clinical differentiation. Imaging studies should only be used to confirm tumour location after rigorous biochemical tests. Surgical management remains to be the definitive cure for most of the cases, hence prompt diagnosis and localization is necessary.

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P543

Hyperparathyroid jaw tumour syndrome

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Hyperparathyroid jaw tumour syndrome is a familial form of primary hyperparathyroidism. Individuals are predisposed to develop parathyroid carcinomas (15%), ossifying fibromas of mandible and maxilla (30%), renal abnormalities including cystic lesions and hamartomas, and uterine tumours (1,2). The pathogenic mutation is in CDC73 gene (previously known as HRPT2 and C1orf28) inherited in an autosomal dominant manner.
Our patient was the first person in the UK to have the diagnosis confirmed on genetic testing. Two sisters had primary hyperparathyroidism. Father had hyperparathyroidism and an ossifying fibroma. During subsequent follow up, she was found to have a small neuroendocrine tumour of her pancreas and is under regular review. The proposed screening protocol is discussed.
References
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P544

Multimodal combination of interferon and loco-regional treatment for disease control in progressive metastatic pheochromocytoma/ paraganglioma patients

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Interferon-α (IFN-α) has shown some activity in neuroendocrine tumors with disease stabilizations. Malignant pheochromocytoma and paraganglioma (MPPGLs) have a heterogeneous behavior with a slow progression rate, most of the time and a high frequency of bone metastases. Stabilizing disease and preventing skeletal-related events are two goals to achieve in the management of MPPGLs patients.
This retrospective study evaluated a multimodal strategy of IFN-α combined to loco-regional treatments (LRT) (radiation and/or interventional radiology and/or surgery) for disease control in patients with progressive MPPGLs. Progression free survival (PFS) was primary endpoint; response rate, safety and symptomatic efficacy were secondary endpoints.
Eleven consecutive patients received peg-IFN-α 2A (90–180 μg/week) or IFN-α 2B (1.5–3 MU×3/week) at our institution between December 2005 and May 2010 as first ($n=3$), second ($n=5$) or subsequent line ($n=3$) of treatment. Six patients were men (55%); median age was 41. At the beginning of treatment, ten patients had progressive disease demonstrated by PET scan ($n=8$), MIBG ($n=3$) or CT-scan ($n=2$); data were missing for one patient. Nine patients had bone-predominant disease (bone-only, $n=4$). During IFN-α therapy, a mean number of 3 (range 1–8) bone directed LRT were performed. Most frequent all grade IFN-α-related toxicities were asthenia ($n=9$), anemia ($n=5$), lymphopenia ($n=5$), diarrhea ($n=3$). One patient had cardiac arrest while on therapy and survived. Symptomatic relief of pain, headaches, diarrhea or sweating occurred in 43% of seven symptomatic patients. Response was evaluable in ten patients: one partial response, eight stable diseases and one progressive disease (PERCIST or RECIST1.1) were seen. With a median follow up of 54 months, median overall survival was not reached and median PFS was 14.4 months.
This study demonstrates the symptomatic and stabilizing effect of a multimodal treatment combination of IFN-α and LRT in progressive metastatic PPGLs.

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P545

The metabolic complications in adrenal tumors: a retrospective study in 56 patients

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Introduction
The metabolic complications in adrenal tumors, regardless secretor or not, are a common finding, thus representing a supplementary warning in these patients related to the non-endocrine therapy as anti-hypertensives, or hypolipemiants drugs, etc.
Aim
We analyze the frequency of metabolic complications in primitive adrenal tumors.
Materials and methods
This is a retrospective study in patients diagnosed with adrenal tumors (secretor or non-secretor tumors). The patients with Cushing's disease were not included, regardless the adrenal aspect. The histological confirmation was obtained in all the secretor cases and only in 19% of non-secretor adrenal tumors (incidentalomas) where surgery was not performed. This is a retrospective study.
Results
Fifty-six patients were registered. 14.28% of cases had bilateral tumors. The mean age was 48.2 years. 12.5% of patients were men. 8.9% of patients had adrenal Cushing.12.5% had pheocromocytoma. 14.28% had adrenal cancer. 57.1% had non-secretor adrenal tumors. The others had rare diagnosis as gangliocytoma or schwannoma. All the patients with phecromocytoma and adrenal Cushing had different types of arterial high blood pressure and 56.2% of the patients with incidentalomas (essential hypertension). 60% of patients with adrenal Cushing had diabetes mellitus, 28.57% of those with pheocromocytoma, and 21.8% of those with incidentalomas. 80% of patients with adrenal Cushing were non-normal weighted. 65.62% of the patients with non-secretor tumors were obese or over-weighted. 80% of patients with Cushing syndrome had high blood cholesterol, and 42.85% of those with pheocromocytoma, and 46.87% of those with incidentalomas. We found no correlations between the metabolic component and the tumor size as pointed by computed-tomography, or the hormonal levels in secretor tumors.
Conclusion
In our study, the metabolic complications were found in more than half of the patients, regardless the secretor profile but the relationship to the adrenal hormones production is not presented in non-secretor tumors, thus an essential overlapping component as metabolic syndrome is associated.

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P546

Malignant melanoma and prolactine imbalance

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Introduction
Malignant melanoma is a challenging illness for researchers due to unknown hormonal disorders involved in promoting/developing this disease. Some authors accept nowadays, that prolactin is involved in extrapituitary carcinogenesis.
The authors aimed to study the status of prolactin in patients with melanocytic lesions and the impact of surgical removal on prolactinemia.
Method
The study included 128 adults with melanoma and 48 with dysplastic nevi. All the patients were evaluated before, and after surgical removal of the tumor. The control group involved 48 healthy participants. All the groups were homogenous for age and sex.
Prolactin (chemiluminescent method by access immunoassay systems) was evaluated at diagnosis (preoperative) and 8 weeks after surgical removal.
Results
High levels of prolactin were determined in patients with melanoma comparative with prolactin in patients with dysplastic nevi (10.55 ± 8.50 vs 5.94 ± 2.87 ng/ml 95% CI, $P<0.01$) and in the control group (10.55 ± 8.50 vs 5.74 ± 3.66 ng/ml, 95% CI, $P<0.01$). The statistical analysis showed no significant correlations between prolactin variation and tumor characteristics (site tumor, histological type, presence/absence of ulceration, Clark level, Breslow index).
Prolactin variation before and after the surgical removal was statistically significant (10.55 ± 8.50 vs 9.23 ± 5.43 ng/ml 95% CI, $P<0.01$), while prolactin did not vary significantly in dysplastic nevi group.
Conclusions
High levels of prolactin were detected in patients with melanoma, levels that decreased after surgical removal of tumor. These results sustain the idea that prolactin is an active participant in tumor development. The data of our study could permit the development of new therapeutic targets that can block the effect of prolactin by decreasing the local production of prolactin, or by blocking its receptors.

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P547

Influence of iron deficiency on angiogenesis in melanoma patients

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Introduction
Iron deficiency contributes to stabilization of HIF1α and upregulation of genes that stimulate angiogenesis in diseases associated with oxidative stress. The authors' interest is centered on evaluation of the relation between iron status and angiogenesis (VEGF-A, sVEGFR) in patients with melanoma.
Methods
The study included 128 patients with melanoma before surgical removal of the tumor. We determined ferritin (immunoturbidimetric method), sideremia (spectrophotometric method), transferrin (immunoturbidimetric method), transferrin saturation coefficient for evaluating iron status in organism, and, VEGF-A, VEGF-R (ELISA method) for evaluating angiogenesis.
Results
Iron deficiency was identified in 31 (24.2%) melanoma patients and was defined as follows: sideremia <30 µg/dl, ferittin <20 ng/ml, transferrin saturation coefficient $<15\%$. The authors observed no changes of iron status in 85 (66.4%) melanoma patients. Iron overload, serum iron >150 µg/dl, ferritin >200 ng/ml, transferrin saturation coefficient $>50\%$, was identified in 12 (9.3%) melanoma patients.
The statistical analysis showed a strong correlation between ferritn level and VEGF: $r=0.823$, IC$=95\%$, $P<0.001$, between feritin level and sVEGFR1:-VEGF-A ratio: $r=-0.362$, IC$=95\%$, $P<0.05$ in melanoma patients with iron deficiency. This relation was not observed for other studied situations (melanoma patients with normal status of iron or iron overload).
Conclusions
Low serum levels of iron were associated with high concentrations of proangiogenic mediators (sVEGFR and VEGF-A) in melanoma patients, factor that could increase tumor angiogenesis.

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P548

Calcitonin comparison study of LIAISON XL vs IMMULITE 2000

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Introduction
Automated calcitonin assays are one-step sandwich chemiluminescence immunoassay, intended for the quantitative determination of calcitonin in human serum. Calcitonin is the most specific and sensitive marker of medullar thyroid carcinoma for both primary diagnosis and the post-surgical follow-up. Recently a new fast and high throughput Calcitonin II-Gen assay developed on the fully automated LIAISON instruments, which is suitable for high working capacity laboratories.
Design
Evaluation took place in Clalit HMO central lab, currently using the SIEMENS IMMULITE® 2000 Calcitonin assay. Forty-one randomly outpatients' serum samples collected from Clalit Central Laboratory, 42 from Clalit Haifa and Western Galilee Laboratory and nine from Clalit Jerusalem laboratory. The samples were tested on LIAISON XL and IMMULITE 2000 in parallel. Results were analysed according to patients' gender and kit performance was tested.

Results
Correlation between LIAISON® Calcitonin II-Gen and IMMULITE® 2000 calcitonin was analysed for: i) samples from Clalit Atidim central laboratory. ii) Samples from Clalit Haifa and Western Galilee Laboratory. iii) Samples from Clalit Jerusalem laboratory. Calculated total clinical agreement was 100, 100 and 89% respectively. Correlation and total clinical agreement were calculated on all three labs' samples showed $r=0.995$, and 99% respectively. Correlation between LIAISON and IMMULITE Calcitonin of NEQAS samples showed $r=0.9995$. Precision of LIAISON® Calcitonin II-Gen was tested between and within runs and found to be 3.75–5.54% CV and 1.24–3.45% CV respectively. Functional sensitivity showed values of 1.136, 1.299 and 2.233 pg/ml. Dilution test showed linearity of $r=0.9922$.
Conclusions
High correlation observed between LIAISON and IMMULITE Calcitonin together with high clinical agreement. It is concluded that the LIAISON® Calcitonin II-Gen can be used as reliable and accurate kit for high throughput laboratories

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P549

Epidermal growth factor pathway as a possible target in the medical therapy of bronchial carcinoids

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Bronchial carcinoids (BC) are rare tumors originating from endocrine cells dispersed in the respiratory epithelium. Currently, the main BC treatment is surgery, that can be curative in most of the cases, but is not feasible for large, infiltrating and metastatic disease. In these settings, medical therapy is often tried, being mainly represented by chemotherapy and radiation in the attempt to reduce tumor mass, while somatostatin analogues are employed for symptomatic control. Therefore it is important to identify new therapeutic targets and new molecules capable of providing adequate medical treatment for patients with BC for which surgical removal is not feasible. Growth factors which are important in experimental models of neuroendocrine tumors include epidermal growth factor (EGF), transforming growth factor (TGF) α, TGFβ. EGF and TGFα bind to the EGF receptor to stimulate the PI3K/RAS/RAF/MAPK pathway, leading to the transcription of genes associated with cell proliferation, invasion and metastasis. Our aim is to evaluate the effects of Sunitinib, a multi-targeted receptor tyrosine kinase (RTK) inhibitor, and NVP-BEZ235, a PI3K/mTOR inhibitor, on human primary BC cells cultures in order to verify the involvement of the EGF pathway in regulating crucial cellular processes.
Human BC primary cultures were treated with Sunitinib or NVP-BEZ235, alone or in combination with EGF. EGFR expression, cell viability and caspase 3/7 activation were evaluated.
By immunofluorescences we found that EGFR is expressed in all primary cultures. In addition, 100 nM NVP-BEZ235 and 10 μM Sunitinib inhibit cell viability by 30 and 20% ($P<0.01$), respectively. Both NVP-BEZ 235 and Sunitinib promote apoptosis (100%). 100 ng/ml EGF impairs the antiproliferative and pro-apoptotic effects of both Sunitinib and NVP-BEZ 235.
These data suggest a possible role for EGFR pathway as molecular target in the medical treatment of BC. Further studies are necessary to understand the molecular basis of this mechanism.

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P550

Steroid-induced psychosis as a manifestation of ectopic ACTH secretion from metastatic poorly differentiated neuroendocrine tumour

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Case report
A 65-year-old fisherman presented with altered mental status, agitation, generalised oedema, dyspnoea, liver impairment and severe persistent hypokalaemia (2.2 mmol/l). History: COPD, IPF (on steroids since May 2012), ex-smoker. Physical examination: deeply tanned, Cushingoid facies, severe proximal myopathy. Medications prior to admission: prednisolone 40 mg OD, furosemide 40 mg OD, inhalers, and lansoprazole. In a view of clinical and biochemical features of steroid access, the Endocrine referral was made.
Patient had the following abnormal endocrine tests: cortisol – 3118 nmol/l, ACTH – 632 ng/l, total chromogranin A – 480 μ/l, 5-HIAA – 551 μmol/24 h. CT thorax/abdomen showed multiple liver metastasis, pulmonary fibrosis, RUL nodule, enlarged paratracheal, subcarinal lymph nodes, bilateral adrenal hypertrophy.
CT haed/MRI of pituitary were normal. Liver biopsy confirmed the presence of poorly differentiated (very high Ki67 index – 90%) small cell neuroendocrine tumour which positively stained for TTF1, CK7, CD56, AE1/3, chromogranin, synaptophysin. Positive staining for TTF1 and CK7 are sought to be in favour of primary lung lesion.
The diagnosis of poorly differentiated metastatic small cell neuroendocrine tumour with ectopic ACTH secretion was made. Patient was started on metyrapone 1 g 4 hourly. Ketoconazole 200 mg OD was added later. His cortisol and LFT had initially improved and potassium normalised. Oncology team put him on 3-month course of carboplatin with etoposide. Further investigations suggested for carcinoid in a form of octreotide scan were not recommended by Oncology Department due to its relative insensitivity in the settings of poorly differentiated neuroendocrine tumour. Patient passed away just in 4 months since presentation despite intensive chemotherapy.
Conclusion
We reported this case because of the interesting clinical presentation of an ectopic ACTH secretion exacerbated by exogenous steroid intake. This case also shows the importance of good differential diagnosis of hypokalaemia. Treatment options were limited due to the aggressive nature of the tumour.

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P551

Adrenal incidentalomas – a retrospective analysis for the period 2001–2011

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Introduction
With the wider availability of imaging techniques there is an increasing number of newly diagnosed, accidentally found pathological formations in the adrenal glands known as adrenal incidentaloma (AI). Currently, the professional consensus is that hormonally active incidentalomas and those suspicious of malignancy should be indicated for surgery.
Methods
In our retrospective study, we analyzed data of 141 patients with a total of 160 AIs, who were followed up in 2001–2011 by the Outpatient Endocrinology Department of the Military University Hospital in Prague. Most AIs were up to 3 cm in size (77.5%), 11.25% had a size of 4–6 cm, and 11.25% were above 6 cm in size. Adrenalectomy was primarily indicated in all patients with AI greater than 6 cm. During the follow-up period, 12 AIs increased in size by 1 cm and four AIs by 2 cm or more. Hormonal activity was examined in all patients. Pathological hormonal overproduction was detected in 24 patients. During the 10-year follow-up period, a total of 36 patients underwent surgery, 21 because of the size of AI, nine for endocrine activity of the AI and three patients due to size progression of the AI. The result of postoperative histology was available for 21 incidentalomas. Malignant tumours accounted for 28.5%. All were larger than 6 cm and four were found to be hormonally active.
Results
All malignant tumours were larger than 6 cm. There is no doubt about the need to indicate adrenalectomy in these patients. Size progression of the AI had always been recorded already during the first 2 years of the follow-up. In patients with growth progression, detected hormonal activity or size 4–6 cm we use an individual approach and long-term follow-up.

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P552

Androgen receptor expression in stromal and epithelial prostate cancer tissue specimens

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Prostate cancer (PCa) is one of the leading causes of tumor death in Western countries. Modifications in expression and functional alterations that involve the

androgen receptor (AR) have been implicated in the progression of PCa and in the development of androgen independence; however, the role of AR in these processes is still debated, as contrasting results have been reported in several studies evaluating the relation between AR expression and disease progression (Tamburrino *et al.*, 2012). Such evaluations are performed in PC specimens where, however, tumor tissue may be mixed to stromal and normal. There is now evidence in the literature that AR role in PCa may vary depending on its location. Indeed, studies performed in animal models (Niu *et al.*, 2008) pointed out the different role of epithelial (protective toward a malignant phenotype) vs stromal (leading to tumor aggressiveness) AR in PCa. The present study was undertaken to evaluate AR, EGFR, PSA and PTEN mRNA expression in stromal and epithelial compartments of PCa specimens following careful microdissection.
So far we have analyzed 130 microdissected samples from 20 patients and further analyses are in progress. Preliminary results indicate that AR expression is correlated to that of EGFR in epithelial ($r=0.98$, $P<0.0001$) but not in stromal compartment. A similar correlation is found between AR and PSA in epithelial compartment ($r=0.67$, $P<0.002$). PTEN expression tends to decrease and to become undetectable in high-grade tumors as expected. AR expression appears to be lower in microdissected carcinoma areas with higher Gleason scores. In few patients with locally invasive tumors AR expression is higher in stromal respect to epithelial compartment.
In conclusion, evaluation of AR expression in microdissected PCa specimens may reveal new insights on the role of the steroid receptor in PCa progression.

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P553

Prolactinoma and vestibular schwannoma: a very rare association

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Introduction
The occurrence of primary pituitary tumour and cerebral schwannoma in the same person is very rare. Only few cases have been reported so far. The mechanism of this association is still unclear.
Aim
Our aim is to report a man with two different brain tumours: a prolactinoma and a cerebellopontine schwannoma in order to discuss the possible mechanism of multiple neoplasms arising in the same person.
Case report
A man aged 50 years, without any history of neurofibromatosis, known to have a right ear malformation, consulted for a sensation of exorbitism of the right eye. Clinical and ophthalmological examinations were normal. But, cerebral MRI showed two tumours: one in the pituitary area and the second in the right cerebellopontine angle.
The first tumour is a prolactinoma: PRL = 1737 ng/ml ($n<15$), tumour size = $28\times29\times24$ mm with a supra sellar extension and infra-sellar invasion filling the sphenoidal sinus. The second measures $18\times16\times22$ mm with high signal intensity on T2 weighted MRIs, strongly enhanced after gadolinium injection suggestive of a schwannoma.
Conclusion
This association must be known as a new field of multiple neoplasms arising in the same person whose mechanism is still debated, especially in people without any personal history of radiation and neurofibromatosis.
Key Words
Vestibular schwannoma, pituitary tumour, prolactinoma.

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P554

The adverse effects in sub-optimal mitotane doses: a retrospective study

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Introduction
There are many side effects caused by mitotane and several are registered at drug levels lower than therapeutical doses.
Aim
We observed the mitotane side effects in patients treated for adrenal cancer.
Materials and methods
This is a retrospective study in adrenal tumors.
Results
Out of 56 patients included in a database with adrenal tumors, we found eight patients (14.25%) with adrenal cancer and histological confirmation of the disease. The mean age was 53.57 years. Twenty-five percent of patients were men. 62.5% of these patients were treated with mitotane and 25% of them with chemotherapy. All patients with adrenal disease had unilateral adrenal tumors (50% of them were on the left side). Twenty-five percent of patients did not have metastasis at the moment of adrenal surgery. The mitotane was started after the adrenal surgery from 2 months to 2 years. All the patients had adrenal insufficiency immediately after surgery because of the previous Cushing disease caused by the tumor itself, so this was not registered later as a side effect to mitotane. The adverse reactions to mitotane were registered from very low doses were mild digestive effects as nausea were registered in all patients treated with mitotane from the first days. One female patient had multiforme erythema 10 days after starting the therapy. After temporary arrest of the mitorane, the drug was re-initiated and no erythema was found. Sisty-six percent of patients treated with mitotane had hypercholesterolemia at a level under 3.3 mg/dl. At least then 6 months from starting the therapy 33% of patients had peripheral neuropathy, anemia, and the men hypogonadism and painful gynecomastia. All these reactions were registered at very low doses of mitotane, far from therapeutical window of efficiency. No correlations of the side effects were found to the initial size of the tumor, to the severity of the clinical phenotype before adrenalectomy, or to the Weiss score.
Conclusion
The mitotane in adrenal cancer is a useful drug but numerous adverse reactions are registered from the beginning of the therapy, thus close follow-up is necessary.

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P555

Type 1 gastric endocrine tumors as an autoimmune disease, with emphasis to lymphocytic thyroiditis

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Introduction
Type 1 gastric endocrine tumors (T1-GET) incidence is increasing world while mainly due to widespread use of upper endoscopy. Autoimmune disease (AID) is the hallmark of T1-GET, pernicious anemia is often found at presentation. Association with lymphocytic thyroiditis (LT) has been described.
Aims
Retrospective evaluation of GET data from patients (pts) followed at IPO-Porto, including the presence of other AID with emphasis to LT.
Patients and methods
Retrospective data from 48 GET patients files followed at Endocrine Tumors Clinic of IPO-Porto was reviewed, 20 pts were excluded because of insufficient data or diagnosis before 2006, because at that time classification was not performed. Age, gender, normal gastric mucosa (NGM), classification, stage, treatment, presence of pernicious anemia (PA), LT and other AI diseases; parietal (p-ab), intrinsic factor (IF-ab) and thyroid antibodies (TPO-ab, TG-ab), thyroid function (TF) and survival were evaluated.
Results
Age 58.7 ± 10.2 years; F/M: 3/1; T1 = 85.7%, T2 = 0%, T3 = 14.3%. Median gastrin = 1107.6 ± 842.7 pg/ml. Two of four T3 were metastatic. T1-NGM showed atrophic gastritis (85.7%), intestinal metaplasia (61.5%) and HP positivity (15.8%). 85.7% (100% T1-GET) were submitted to endoscopic treatment. AID was identified in 71.4%: PA 93.4%, vitiligo 1 pt, another has lupus erythematosus (LE) and Crohn's disease(CD). Positive PC-ab 63.2% and FI-ab 5.6%. LT was identified in 33.3%. TPO and Tg-ab were positive 22.4 and 11.1% respectively. Insufficient data concerning other DAI was found in 28.6–31.4%. 21.1% pts had either clinical or subclinical hypothyroidism. Thyroid nodules (TN) were diagnosed in 50% of pts who were submitted to US neck ($n=6$). Three of four of T3 pts have died, 100% of T1 were alive.
Conclusion
As in other series, most of our GET pts are type1. In this group there was a high proportion of AID, including PA, vitiligo, LE and CD. One third of T1-GET had LT. Among these, one fifth had abnormal function tests and half of pts who were submitted to neck US had TN. All T1-GET must be evaluated in order to exclude association with other AIDs, especially LT. Prospective studies should be performed in other to increase our knowledge about this entity.

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P556

Acromegaly, primary hyperparathyroidism and meningioma – an unsual association in an asymptomatic patient with MEN1 syndrome

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Introduction
MEN1 is inherited as an autosomal dominant trait, with a prevalence of 2–20/100 000 in the general population. It is characterized by tumors envolving the parathyroid glands, the endocrine pancreas and the pituitary. Other tumors are more rarely associated: adrenal adenomas, carcinoid tumors, pheocromocytomas, angiofibromas, lipomas, collagenomas and meningiomas. The presence of two or more of MEN1 associated tumors is diagnostic of the syndrome.
Case report
A 56-year-old man presented to the Endocrine consultation for investigation of asymptomatic multinodular goiter, with benign features in the neck sonogram. At physical examination the physician detected mildly enlarged hands and coarse facial features. The patient had been diagnosed with primary hypertension and kidney stones 2 years before. His medical history was otherwise irrelevant. Family history was negative for endocrine disease. The endocrine evaluation revealed mildly increased GH (3.3 ng/ml) and IGF-1 (704 ng/ml). The remaining anterior pituitary hormones and prolactin were normal. Throughout the oral glucose tolerance test, GH levels were equal or above 3.0 ng/ml, confirming the diagnosis of acromegaly. The cranial and sellar MRI revealed a left sellar mass 15×7 mm in diameter, inducing slight deviation of the pituitary stalk, indicating the presence of a pituitary adenoma. There was also a voluminous extra-axial left frontal tumor (43×37 mm) suggestive of a meningioma. Serum levels of calcium (11.3 mg/dl) and PTH (116.3 pg/ml) were increased, with normal renal function and 25OH vitamin D levels. Primary hyperparathyroidism (PHPT) was diagnosed. Insulinemia, gastrinemia and abdominal CT were normal. The results of the genetic test of the *MEN1* gene are not yet available.
Conclusions
The phenotypic manifestations of acromegaly were the first clues to the diagnosis of MEN1 in this asymptomatic patient. The unsual association of acromegaly, PHPT and meningioma raised suspicion of a causative common genetic mutation and is diagnostic of MEN-1 syndrome.

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P557

Aggressive adreno-cortical carcinoma (ACC) associated with two rectal tumors (adenocarcinoma and neuro-endocrine) and somatic Kras mutation without microsatellite instability: is there a link?

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Aggressive adreno-cortical carcinoma (ACC) is a rare, aggressive malignancy, with poorly understood molecular pathogenesis. As a result, therapeutic options are currently limited, surgery being currently the lone curative modality. Most cases of ACC are sporadic, although some familial cancer syndromes (Li–Fraumeni, Beckwith–Wiedemann, MEN1, Carney complex, congenital adrenal hyperplasia, etc.) are associated with an increased incidence of ACC. The genes involved in these syndromes, the adrenocorticotropic hormone-cAMP-protein-kinase-A and the β-catenin gene (*CTNNB1*), which lead to constitutive activation of Wnt signaling, are also implicated in ACC tumorigenesis. Understanding these pathways should lead to targeted therapy such as IGF, mTOR, steroidogenic factor-1, and MDR1 antagonists. Besides, rare cases of ACC are associated with colon cancer, sometimes associated with *APC* gene mutation and Lynch syndromes. We report a new case.
A partially deaf 43-year-old woman, with a narrow face, was diagnosed with ACC in reason of a painful 12 cm heterogeneous adrenal mass, secreting cortisol and androgens with angio-invasion impeding surgical resection. PET-FDG showed adrenal, lumbar and rectal uptake. Rectal biopsies showed a well-differentiated adenocarcinoma and a moderately-differentiated neuro-endocrine tumour. Rapidly occurring liver metastasis were in favour of poorly-differentiated ACC. The patient's father had died from colonic perforation and her son had ulcerative colitis. Kras gene analysis on the colorectal carcinoma showed a somatic mutation (exon 2,V12G), but no microsatellite instability (phenotype MSS), rebutting Lynch syndrome. Kras and TP53 analysis in blood and liver metastasis are under investigation.
In conclusion, i) the association of two colorectal cancers with aggressive ACC is a particular challenge to define the priority of treatment between these two tumours; ii) the somatic Kras mutation inducing activation of the Ras/Raf/MEK/ERK pathway occurs in 40% of colorectal cancer but its role in ACC remains unknown. The involvement of Kras mutations in ACC could lead to new therapies such as Kras or PIK3 kinase inhibitors.

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P558

Patient-specific management of paragangliomas

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Introduction
Paragangliomas (PGLs) are extra-adrenal, neural crest-derived neuroendocrine tumours. There are sympathetic (usually abdominal and thoracic regions) or parasympathetic PGLs (head and neck). Catecholamine excess usually occurs in sympathetic PGLs.
Case
A 34-year-old lady initially presented with left-sided neck swelling. She was diagnosed with left cervical PGL which was excised. Twenty years later, she noticed another lump in the same region and functional imaging indicated a second primary disease and underwent repeat surgery. Subsequent genetic testing revealed the succinate dehydrogenase enzyme D (SDHD) mutation. Her sister was also diagnosed with cervical PGL. Two years after surgery, she described daily intermittent forceful palpitations and was hypertensive with BP 170/95. Urinary catecholamine collections, plasma normetanephrine and metanephrine levels were normal. She had anatomical CT and MRI imaging, octreotide isotope scan and MIBG scintigraphy. Functional imaging revealed multiple sites in her mediastinum and her case was discussed at the regional neuroendocrine tumour meeting. As she was intolerant of phenoxybenzamine, doxazosin was started followed by propranolol. She had surgical removal of the right cervical, subclavian and aortic root PGLs. Recently, she was admitted with central chest pain associated with elevated cardiac enzymes. ECG demonstrated lateral ST depression, and an echocardiogram showed mid to apical, segmental and lateral wall hypokinesis with apex ballooning. Coronary angiography revealed normal arteries. Repeat echocardiogram showed ventricular function had returned to normal. A diagnosis of Takotsubo cardiomyopathy was made. Urinary catecholamine, plasma normetanephrine and metanephrine levels were normal throughout.
Conclusions
Head and neck PGLs are usually non-functioning and present as space-occupying symptoms. It is unusual she had symptoms of catecholamine excess. Functional imaging proves invaluable and this highlights the need to discuss complex cases in multidisciplinary meetings. With reported associations between Takotsubo cardiomyopathy and catecholamine-producing PGLs and phaechromocytomas, could this episode be a manifestation of her PGL? There should be a low threshold for functional imaging for PGLs in patients with familial genetic mutations.

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P559

Malnutrition cause of Secondary Osteoporosis after surgical operation of Glucagonoma

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Introduction
Glucagonoma is a rare condition with annual incidence 1 in 20 million, associated with diabetes mellitus, dermatitis, deep vein trombosis and depression.
Case report
A 55-year-old woman patient was hospitalized at our department because of intense discomfort of the skin lesions that were pruritic and painful, erythematous area of skin with blisters that breach after a few days, red tongue, cracks on the mouth corners. She had a constant weight loss accompanied with bloody diarrhea.

Her weight was 36 kg, height 150 cm and her BMI was 16. We made 75 g OGTT and it was normal. She was misdiagnosed like contact dermatitis magnum et pedum, stomatitis protetica, erythema exsudativum multiforme, colitis. On the examination she had cheilitis angularis, atrophic glossitis, stomatitis, normochromic normocytic anemia and the Hct was 0.27, SE 70/100. She had dermatological changes – erythematous patch, blisters centrally, erodes, crusts, heals with hyperpigmentation. We made a lab test and we got that her glucagonemia was increased twice than normal. The normal values are 200 ng/l and she had 400 ng/l. We made a CT scan where a round form of a tumor was noticed in the pancreas with dimensions 5 cm width and 8 cm length. After that, she underwent a surgical operation and the surgeon made a distal splenopancreatectomy to remove the tumor. The tumor immunohistochemistry was positive of glucagon, synaptophysin and chromogranin-A. After the operation, she suffered from malnutrition and she came again at our department to check herself for osteoporosis. We made a DXA scan and we saw that she had a generalized secondary osteoporosis caused due to malnutrition after operation (T score $= -4.0$ on the spin, T score $= -3.8$ on the right hip and T score $= -3.6$ on the left hip).

Conclusion

Long-term misdiagnosed glucagonoma explain appearance of other co-morbidites such as osteoporosis and anemia.

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P560

Clinical, biochemical, genetic and histological features of composite pheochromocytoma/ganglioneuroma adrenal tumors: a series of seven cases from two French academic centres

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Introduction

Adrenal pheochromocytomas have the same embryonic origin, i.e. the neural crest, as peripheral neuroblastic tumors such as ganglioneuromas, ganglioneuroblastomas and neuroblastomas. Ganglioneuromas are benign and silent tumors in that they usually do not secrete catecholamines in contrast to pheochromocytomas. Rarely, they can associate with pheochromocytomas to form composite tumors.

Patients and methods

We have retrospectively studied seven patients with pheochromocytoma/ganglioneuroma composite adrenal tumor followed up in two French academic departments of endocrinology. The clinical, biochemical, genetic and histological characteristics of the tumors were collected. In addition, immunohistochemical labelling of tumor slices with specific antibodies directed against the key enzyme for adrenaline synthesis phenylethanolamine *N*-methyltransferase (PNMT) were carried out in order to better individualize the two components of the neoplasms.

Results

In all cases, association of the adrenal mass with clinical and biological signs of catecholamine excess led to the initial diagnosis of pheochromocytoma followed by adrenal surgery. The ganglioneuroma components of the neoplasms were identified at pathological examination of the tissues. Four patients carried germinal mutations affecting the NF1 (two patients), KIF1Bbeta (one patient) and MYC-associated factor (MAX; one patient) genes. Five patients harboured associated neoplasias and two patients had subclinical hypercortisolism related to hyperplasia of the adrenal cortex adjacent to the tumors. Immunohistochemical PNMT labellings of tumor sections allowed perfect discrimination of the two tumor tissue components by revealing intense staining of pheochromocytes contrasting with no signal in ganglion cells.

Conclusion

We report a large series of adult adrenal pheochromocytoma/ganglioneuroma composite tumors. In addition, we show that, in addition to routine histological examination of the tumor tissues, immunohistochemical studies with antibodies to PNMT can be helpful for the diagnosis of the disease by facilitating identification of the two tumor components.

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P561

Everolimus treatment in a series of patients with advanced neuroendocrine tumors

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Introduction

Everolimus is an oral mTOR inhibitor that exerts antineoplastic effects inhibiting cell proliferation, survival and angiogenesis. Its activity in advanced neuroendocrine tumors (NETs) has been demonstrated in controlled trials and everolimus was approved by the FDA for the treatment of progressive, advanced pNETs in May 2011.

Materials and methods

We treated with everolimus, at the dosage of 10 mg once daily, 14 patients with advanced, progressive, low- or intermediate-grade NETs for a mean period of 11 months. Somatostatin analogues treatment was continued in all patients. Twelve of 14 patients had previously undergone peptide receptor radionuclide therapy (PRRT) with either Lutetium or Yttrium.

Results

According to RECIST criteria, stable disease was observed in 9/14 patients and partial response was achieved in 2/14 patients. Median progression-free survival was 12.0 months. Drug-related adverse events included stomatitis (7/14), hyperglycaemia (7/14), hypertriglyceridemia (5/14), pneumonitis (4/14), hematologic toxicity (4/14), peripheral oedema (4/14) and rash (2/14). Grade 3 and 4 adverse events included pneumonitis (three cases) and thrombocytopenia (two cases). Dose reduction was required in 5/14 patients.

Conclusion

Our data confirm the efficacy of everolimus in the treatment of progressive, advanced NETs. The apparently higher rate of grade 3 and 4 adverse events is probably related to the high proportion of patients in our series that had previously undergone PRRT, as it may enhance everolimus potential myelotoxicity.

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P562

Are hibernoma or lipoma a marker of type 1 multiple endocrine neoplasia (MEN1) aggressiveness?

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MEN1, autosomal dominant, is characterized by combined tumours of the parathyroid glands, pancreatic islet cells, and the anterior pituitary, sometimes associated with other endocrine (adrenocortical, foregut carcinoid) and nonendocrine (lipoma, angiofibroma, collagenoma, ependymoma, meningioma) tumors. It is caused by inactivating mutations of the *MEN1* tumour suppressor gene (chromosome 11q13), encoding menin. Menin, involved in activation of gene transcription, regulates PPARg-dependent adipocyte differentiation (Dreijerink Mol Cell Biol 2009), whereas PPARg is expressed in several MEN1-related tumor types.

We report two cases of unrelated obese male patients presenting with aggressive MEN1 and large either hibernoma or lipoma. Case 1, 39-year-old, without any family history, was diagnosed with Zollinger–Ellison syndrome with high gastrin, pancreatic polypeptide and glucagon level, invasive macroprolactinoma, groin angiofibromas, 5 cm adrenal mass and hyperparathyroidism. MEN1 gene analysis showed two not-indexed heterozygous mutations (exon 2AB: c105G > AA/exon 3: c737_740del). At 43 years old, increasing size of the pancreatic nodules, and pancreatic and right thigh PET-FDG uptake led to i) total pancreatectomy (13 neuroendocrine pancreatic tumors (pT2MN1: 11 G1, one G2, one G3)), ii)- left adrenalectomy (Weiss 0), and iii) thigh hibernoma resection. Case 2, 25-year-old, belonging to a MEN1 family (c.1546dupC/pArg516-ProfsX15), was diagnosed with severe hyperparathyroidism (with ectopic parathyroid gland), five pancreatic endocrine tumors (with hyperglucagonemia) and a 13 cm FDG-negative lipoma of the left iliac muscle.

In conclusion, hibernomas are benign tumors with morphological features resembling brown fat. Their PET-FDG uptake raises differential diagnosis issues with liposarcomas and metastasis. As lipomas (Vortmeyer J Natl Cancer Inst 1998), they consistently display cytogenetic rearrangements, involving chromosome band 11q13 (Gisselsson Am J Pathol 1999). *MEN1* display a low expression in hibernomas whereas the expression of genes up-regulated in brown fat (PPARg, UCP1) is high (Nord PNAS 2011). These associations raise the question of the relationship between adipose tissue and cancer genesis or MEN1 aggressiveness.

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P563

Malignant pheocromocytoma: vertebral metastasis 18 years after surgery. The importance of prolongued follow-up

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Introduction
Malignant pheocromocytomas are rare. Metastatic disease may be present at the time of diagnosis or may only be evident after. Prognosis is poor. Currently there is no effective cure.
Case report
We describe the case of a 66-year-old woman with a history of pheocromocytoma submitted to left adrenalectomy in 1992. In the past 10 years, she complained of episodes of dorsal back pain associated with hypertensive peaks and tachycardia. In 4/2010, a dorsal MRI was performed revealing D10 pathologic fracture with moderate spinal compression, compatible with bone metastasis. MIBG-SPECT/CT: increased uptake in D10. Guided biopsy: the lesion was immunoreactive for neuroendocrine markers. Octreoscan was negative. She was admitted at the Endocrinology Department in 8/2010. Analytically: chromogranin-A 112 ng/ml (19–98), urinary metanephrines 219 μg/24 h (25–312), VMA 2.45 mg/24 h (<15), 5-HIAA 5.64 mg/24 h (2–6). She started fenoxibenzamine titrated until 10 mg 2id. In 9/2010, she was submitted to radiotherapy of the lesion (total dose 30 Gy). No adverse effects were reported; back pain was not substantially reduced. Spinal CT after showed lesion stability. No neurological deficit. Analytically chromogranin-A 7.8 nmol/l (<6); normal urinary metanephrines. MIBG-SPECT/CT (2/2011): D10 metastasis maintained with similar degree of activity. In 3/2011, she was submitted to 200 mCi of 131I-MIBG, with no complications. The following MIBG-SPECT/CT showed similar degree of activity in D10 and focus of increased uptake in segment VI of the liver. In 6/2011, she was submitted to D10 kyphoplasty. Abdominal CT showed no image suggestive of secondary lesions. In last evaluation, under fenoxibenzamine 10 mg 2id, the patient had minimal back pain with no limitations in her daily activities. Analytically chromogranin-A 14 nmol/l and normal urinary metanephrines. Genetic study was negative.
Conclusion
This case illustrates the importance of treatment individualization. A combination of radiotherapy, MIBG and kyphoplasty was performed in order to decrease pain and to stabilize vertebral secondary disease. Metastasis diagnosed after 18 years reinforce the need of an accurate and prolonged follow-up.

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P564

Gastroenteropancreatic neuroendocrine tumors: descriptive study in a reference Spanish hospital

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Objective
Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are relatively infrequent. We present a descriptive study of GEP-NETs of a reference Spanish hospital.
Patients and methods
Forty-six patients were diagnosed of GEP-NETs during the period 1993–2011. Data regarding demographic, clinical, analytical, anatomopathological and diagnostic variables were collected. Results are expressed as mean ± SD.
Results
Twenty-seven (58.7%) were men. The mean age at diagnosis was 44.5 (15.5) years. 82.6% were sporadic, multiple endocrine neoplasia type 1 was diagnosed in 15.2% and neurofibromatosis type 1 in 2.2%. Localization was 48.9% pancreatic, 40% gastrointestinal tract, 6.7% bronchial and 4.4% thymic. Incidental diagnosis occurred in 11.4% of the cases, 62.9% presented hormone hypersecretion symptoms. In those with clinical symptoms, the mean time until diagnosis was of 7.4 (12.5) months with a median of 3 months.
At diagnosis 69% of the cases presented distant metastases. The most common site of metastases was liver (100%). The most commonly carried out imaging studies for the primary tumor and metastases was computed tomography (CT) scan 89.1 and 100% respectively. Overall, about 97.7% of the patients underwent surgery most of them with curative intent. 33.8% underwent hepatic transplant. 55.5% were treated with somatostatin analogues, 19.4% with chemotherapy, 7.5% with lutetium and 2.5% with itrium.
After a mean follow-up of 7.2 (5.0) years for pancreatic NET and 8.8 (5.3) years for the intestinal NET, the survival was 81 and 66.7% respectively.
Conclusions
The NETs of pancreatic location are the most common in our series. Metastatic disease is frequent during follow-up and in most NETs is already present at diagnosis. Surgery is the first-line treatment for the NETs regardless of their location. Up to one third of the patients received a hepatic transplant, being this method a real option for those patients with metastases confined to the liver.

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P565

Extraordinary effect of ketoconazole in treatment of ACTH-dependent paraneoplastic Cushing syndrome

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Background
Although the excision of ACTH-producing tumors or adrenal tumors is the principal treatment for Cushing syndrome (CS), pharmacologic treatment has a well-established role. Among various medical agents, ketoconazole (KTZ) has inhibitory effect over 17,20 desmolase, 17α-hydroxylase, 11β-hydroxylase, and 16α- and 18-hydroxylase; moreover, it also inhibits ACTH production and cellular growth, partly because of apoptosis induction.
Case report
A 76-year-old female was admitted with suspected hypercortisolism for further diagnostic tests. Increased serum cortisol levels with loss of circadian rhythm (1290, 1083, 1264, and 942 nmol/l at 0800, 1600, 2000, and 2400 h respectively) increased basal urinary free cortisol (basal 2804–2606 nmol/d), non-suppressible in 2 mg (1050 nmol/d) respectively 8 mg dxm test (2555 nmol/d), with increased ACTH levels (282–251 pg/ml) confirmed ACTH-dependent Cushing syndrome (CS). MRI of the pituitary was normal, CT of thorax and abdomen showed only hyperplastic adrenal glands. Bronchofibroscopy was negative, PET/CT with 18FDG showed pathologic lesion in inferior mediastinum. Patient refused to undergo thoracic surgery, thus KTZ at a dose of 400 mg/d was started. Patient tolerated medical treatment without any side effects, UFC levels decreased to normal levels (86 nmol/d) and good effect of KTZ persisted even after reduction of the dose to 100 mg/d (UFC 43.58 nmol/d). At the present time, patient is on this dose of KTZ 36 months with normalized cortisol status.
Discussion
At a dose of 400–1200 mg/day, KTZ can decrease cortisol production in patients with CS from various etiologies. Reports about paraneoplasic CS indicate complete hormonal response in up to 28% of the cases, no prospective studies have been conducted based on KTZ monotherapy. The efficacy data have been drawn from retrospective studies; here, it is reported that KTZ induced biochemical remission in 50% of the patients varying from 25 to 93%. As shown in our case, KTZ may have extraordinary effect in some patients with paraneoplastic CS.

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P566

An analysis of genotype–phenotype correlations and variable clinical expression in families with multiple endocrine neoplasia type 1

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Multiple endocrine neoplasia type 1 is an inherited syndrome that is caused by a germline mutation in the MEN1 gene encoding a tumour-suppressor protein, menin. Currently, no clear genotype–phenotype correlation has been established between clinical forms and MEN1 gene mutations. The aim of the study was clinical characteristics in relation to MEN1 gene mutation in families with MEN1 syndrome treated in our department. To date, genetic testing including complete sequencing of the coding region (exons 2–10) showed significant changes in four families with MEN1 syndrome. In each family, different type of mutation was found and only one of them was previously described. Clinical characteristics and aggressiveness of the disorder were different in each family. Our mutations were localized in the following exons: 4 (c.790_795dupCTGCAG), 7 (c.945delG), 9 (c.1246_1248delGCC), 10 (c.1393C > T). Loss of heterozygosity in tumour tissue will be studied by microsatellite analysis in the family with mutation in exon 9 to confirm its significance.

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P567

Therapeutic difficulties in elderly patients with insulinoma

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Introduction
It is believed that hypoglycemia in the elderly is a dangerous condition and may result in stroke and death. We observed five female patients with insulinoma and over 20-year-long survival.
Material
The material includes five female with the diagnosis of insulinoma. Patients' age ranged from 78 to 91 years while disease duration ranged from 21 to 34 years.
Methods
In all the patients, the diagnosis of insulinoma was based on glucose, insulin and C-peptide levels in the fasting test, while the presence of focal lesion in the pancreas was confirmed in CT, MRI and EUS.
Results
At the time of diagnosis, mean fasting glucose level was 31.4 mg/dl (±9.6), insulin – 19.5 μIU/ml (±14.2) and c-peptide – 8.2 ng/ml (±4.9). In three patients, disease progression was observed after many years of diazoxide therapy. These women have not been qualified for surgery because of their general status. Alcohol ablation of insulinoma was attempted in three patients, but the relief of clinical symptoms after EUS-guided administration of 5 ml ethanol was obtained in only one patient. In the remaining two women, the procedure could not be performed for technical reasons. These patients had receptor scintigraphy with octreotide and blood glucose levels were evaluated in the test with 100 mg octreotide – significant increase in glucose levels was observed in hours 5, 6 and further on, persisting up to 12 h (mean baseline glucose level 41.7 mg/dl; glucose levels following octreotide administration in subsequent hours – 163, 195, 221 mg/dl). In both patients, long-acting somatostatin analogues were started with good clinical effect.
Conclusion
Ethanol ablation of insulinoma and treatment with somatostatin analogues is an alternative in patients who are not good candidates for surgery and in whom diazoxide therapy is ineffective.

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P568

Therapy with ^{177}Lu marked somatostatin analogues in a case of pancreatic metastatic neuroendocrine tumor

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Introduction
Conventional systemic therapies have limited role in metastasized neuroendocrine tumors (NET). Therefore an increasing role is attributed to ^{177}Lu labeled somatostatin analogues (SA).
Case
Man, 52 years, referred because of NET, diagnosed following liver biopsy for hepatic nodules (liver tissue infiltrated by NET, compatible with metastases). Endoscopic ultrasound revealed pancreatic nodular formation, 40×24 mm; ^{99}mTc scintigraphy revealed foci of hyperfixation in the liver, midline abdominal projection and left kidney upper pole. Serotonin levels were 183.0 ng/ml (40–450), A5HI 3.7 ng/24 h (2.0–10), chromogranin A (CgA) 286 ng/ml (< 134), gastrin 118 ng/ml (< 108), insulin 6.43 μUI/ml (6–30) and glucagon 472 pg/ml (100–190). Patient underwent body-caudal pancreatectomy. Histology revealed well differentiated pancreatic NET, G2, 2–10 mitoses/HFA, Ki67 3–20%, peripancreatic soft tissue, lymphovascular and perineural invasion, lymph nodes metastasis, positivity for Cam52, CgA, synaptophysin and NES. ^{68}Ga-DOTANOC-PET revealed liver, lumbo-aortic, periceliac lymph nodes foci of hyperfixation. Embolization of liver metastases and SA treatment was started. Second ^{68}Ga-DOTANOC-PET revealed new hepatic foci. Because of disease progression therapy with ^{177}Lu-DOTATATE was preformed. ^{68}Ga-DOTANOC-PET after three cycles of ^{177}Lu revealed fewer liver foci and no evidence of adenopathy.
Conclusion
Treatment with radiolabelled SA is a promising strategy in patients with inoperable or metastatic NET.

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P569

A case of pheochromocytoma that recognized as panic disorder before its exact diagnosis

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A 44 year-old female patient visited our department for the treatment of diabetes. Her diabetes was pointed out 1 year before and was resistant to the treatment with oral hypoglycemic agents. From around the same time, she complained of repeated attacks consist of headache, palpitation, sweating and nausea. She had suffered from obsessive compulsive disorder for 20 years and her psychiatrist recognized that these attacks were caused by some psychological problems such as panic disorder. However, neither anti-depressant nor anxiolytic agent was effective to improve her recurrent symptoms. Because her diabetes was resistant to the treatment, abdominal ultrasonography was done. It showed large left adrenal mass (approximately 10 cm in diameter). Plasma levels of cortisol and aldosterone were normal. However, 24 h urine excretions of epinephrine, norepinephrine and total metanephrines were markedly increased (epinephrine 171 μg/d, norepinephrine 934 μg/d and total metanephrines 29.9 mg/d). Scintigraphy with 131I-metaiodobenzylguanidine (MIBG) revealed high levels of accumulation at left adrenal mass. From these results, left adrenal mass was diagnosed as pheochomocytoma. The left adrenal mass was removed surgically and the pathological findings revealed typical characteristics of pheochomocytoma. Both blood pressure and blood glucose levels were normalized after surgery. Furthermore, her recurrent panic attack-like symptoms disappeared.
In conclusion, this case shows us the importance of excluding physical abnormalities before making a diagnosis of panic attack-like symptoms. Among a number of medical conditions mimicking symptoms of panic attacks, endocrine disorders such as pheochromocytoma and hyperthyroidism especially should be taken into consideration because they are unnoticeable if not to be careful enough. Many symptoms caused by pheochromocytoma highly resemble to those of panic disorder, but new-onset diabetes could be useful clues in making proper diagnosis. Furthermore, our patient did not complain of intense anticipatory anxiety and agoraphobia which are characteristic of panic disorder. These could also be useful findings to get exact diagnosis.

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P570

Various neuroendocrine tumors in a multiple endocrin neoplasia type 1 family with the same genetic background

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Introduction
Multiple endocrine neoplasia (MEN) type 1 is a rare congenital disease with genetic background. The MEN-1 gene encodes menin protein, that acts as a tumor suppressor. Mutation of one allele and the inactivation of the other allele of this gene lead to clonal proliferation and to the development of tumors. The clinical manifestation of MEN type 1 is a combination of endocrine (parathyroid adenomas, entero-pancreatic neuroendocrine tumors, pituitary tumors) and non-endocrine tumors.
Case report
A 38-year old male patient's investigation was initiated by diffuse disturbances of bone metabolism. Diagnostic procedures revealed presence of primary hyperparathyroidism due to a parathyroid adenoma associated with a pituitary adenoma, a malignant pancreatic neuroendocrine tumor, neuroendocrine cancer of the thymus, hormonally inactive bilateral adrenocortical adenomas and a non-endocrine tumor (facial angiofibroma). The co-existence of all these components witnessed for the diagnosis of MEN type 1. The parathyroidic, pancreatic neuroendocrine and the thymic tumors were surgically removed. For his hyperprolactinaemia and the pancreatic and thymic tumors, he has been kept under continuous bromocriptine and somatostatin analogue therapies, simultaneously. The MEN-1 mutation screening proved a new stop codon mutation: CAG7TAG leading to early stop of protein synthesis. One of the two daughters of the patient was also positive for the same mutation, however, she has not presented any clinical symptoms so far. Their genetically positive older brother (18 years) suffered from hypoglycaemias due to pancreas neuroendocrine tumor. In addition he has hyperparathyroidism that has not yet been operated.
Conclusion
In the present family history, the index patient showed all the major components of MEN type 1. With the aid of genetic screening, the clinical diagnosis was confirmed and the affected family members have been identified. Early diagnostics, continuous observation and initiation of proper therapeutical approaches for their presumed MEN-1 associated tumors became possible.

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P571

Visceral and tumour abormalities in subjects with acromegaly

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Introduction
Acromegaly is a relatively rare disease with numerous complications. Our aim is to look for visceral abnormalities and tumour development in subjects with GH and insuIin like GH (or IGF1) excess.
Subjets and methods
It is a retro-and prospective study that takes in account 112 patients at diagnosis, with pur pituitary tumours secreting GH, or mixed tumours secreting prolactin and GH diagnosed between 1980 and 2012. They all were questioned and examined. They also had heart (clinical, ECG and echosonography), pulmonary (clinical ± polysomnography), gastro intestinal (coloscopy, abdomen ultrasound (US)), thyroid (US ± scintigraphy), prostatic (US, total and free prostatic acid phosphates: $n = 16$), and bone (standard radiographs, bone mineral density) explorations.
Results
High blood pressure was observed in 31.53%, cardiomyopathy in 33.65% and sleep apnea (SA) = 15%. Severe bone abnormalities were seen in 12.12%. For organomegaly, we observed 75% benign prostatic hypertrophy, 40% goiters, 13% splenomegalies and 7.89% hepatomegalies. Concerning neoformations, we observed 7.89% colonic polyps and 2 (1.7%) thyroid cancers.
Conclusion
In this group, cardiovascular complications, prostatic hypertrophies, and organomegalies are as frequent as in literature reports. For SA our results are certainly under estimated. For colonic polyps and malignant tumours our results seem to be low compared to some authors' results.

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P572

The clinical significance of tumor suppressor gene methylation, expression in nodular thyroid disease

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Objective
The tumor suppressor gene methylation lead to gene silencing, it plays an important role in thyroid tumor occurrence and development. This study examined the tumor suppressor gene (PTEN, RASSF1A, DAPK, RARβ2) DNA methylation and mRNA expression status in the peripheral blood of patients with nodular thyroid disease. Discuss its clinical significance. For the benign and malignant thyroid nodules early identification of molecular diagnosis is based.
Method
Select in September 2007–December 2011 a total of 200 cases of nodular thyroid disease diagnosed (including 66 cases of patients with nodular goiter, 50 cases of thyroid adenoma, 24 cases of thyroid cancer and 60 cases of normal control group as a research object, mining fasting venous blood, by the specificity MSPPCR methods to detect PTEN, RASSF1A, DAPK and RARβ2 methylation, by RT-PCR method to detect the peripheral blood of the four genes mRNA expression using SPSS 13.0 statistical software.
Result
In the high methylation status of DAPK, RARβ2, PTEN, RASSF1A gene in the peripheral blood of thyroid cancer, adenoma and nodular goitre patients, and to compare the methylation rate of thyroid cancer > adenoma > nodular goiter. Tip the four gene promoter methylation is common in molecular biological events in the peripheral blood of thyroid tumors. Closely related to the occurrence and development of thyroid tumors. The four genes mRNA expression in the peripheral blood of thyroid cancer and adenoma reduce or even missing, and was negatively correlated with four gene methylation. Tip four genes promoter methylation may be one of the reasons causing gene expression to reduce the missing. The four genes promoter methylation and mRNA expression is realated with lymph node metastasis in thyroid cancer patients. No relationship between nodule size, calcification and ECT.
Conclusion
MSP and RT-PCR method combined detection of nodular thyroid disease in peripheral blood of tumor suppressor gene RARβ2, of DAPK and PTEN, RASSF1A gene methylation and expression.For early malignant and benign thyroid nodules to identify the diagnosis. Determination of MSPDNA and RNA in peripheral blood play an important role in tumor early diagnosis, cancer screening and other aspects.

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P573

MEN2A syndrome: case presentation

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We presented the case of a 20-year-old woman which has presented for endocrinologic evaluation after right adrenalectomy for pheochromocytoma. From her heredocolateral antecedents, we retain her grandmother and sister of her father with sudden cardiac death at early age. At this presentation, she had normal plasma and urinary MN and NMN. Plasma Cg A and Serotonin are normal and the CT exam showed right adrenalectomy and right renal microlitiasis. Genetic analysis showed mutation of the RET gene: TgC634Tgg (Cys634Trp). Ultrasound of the thyroid showed two hypoecogen nodules: 0.52/0.62 cm and 0.53/0.44 cm with irregular borders and with central and periferic vascularization, without laterocervicale lymphadenopathy. TSH, free T4, and ATPO were normal. Serum calcitonin was rised: 21.9 pg/ml (normal: 1.0–4.8 pg/ml). PTH and serum calcium were normal. We decided total thyroidectomy with radical cervical lymphatic dissection and genetic screening for family which showed the same mutation of the gene RET to her father who at the time of diagnosis presented medular thyroid carcinoma.
In conclusion, ussualy, in MEN2A syndrome, medular thyroid carcinoma is the first diagnosed but in our patient the first diagnosed was pheocromocytoma. In this case, long-term follow-up is necessary for detection of metastases and primary hyperparatirodism. Plasma and urinary MN and NMN will be done twice a year, biochemical screening for pheocromocytoma will be done

before any surgery, plasma calcitonin twice a year and if it is rised PET-CT for detection of metastasis for medular thyroid carcinoma.

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Female reproduction

P574

Subcutaneous adipose tissue distribution and metabolic parameters in lean women with polycystic ovary syndrome

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Background
The objective of the study was to compare distributions of subcutaneous adipose tissue and metabolic parameters in lean women with PCOS and lean healthy women.
Materials and methods
PCOS women (according to Rotterdam criteria, mean age 26.54 ± 3.65 years) and 70 healthy control women (mean age 27.84 ± 3.63 years) were investigated in Vilnius city (Lithuania) in 2009–2011. A skinfold caliper device was used to measure skinfolds thickness in 13 sites (biceps, triceps, midaxillary, subscapular, chest, abdominal, suprailiac, thigh, knee and calf). Height, body mass and waist circumference were measured. Bioelectrical impedance analysis using Genius 220 was performed to measure fat mass. Glucose, insulin, C-reactive protein (CRP) and lipids tests were assessed by standard techniques.
Results
BMI (21.10 ± 2.02 vs 21.05 ± 1.65 kg/m^2, $P=0.876$) and waist circumference (69.03 ± 4.94 vs 68.73 ± 4.44 cm, $P=0.689$) did not differ between PCOS and control groups.
Fat mass in lean women with PCOS was higher by 8.92 kg than in controls ($P<0.0001$). In women with PCOS arms' skinfolds (triceps by 2.42 mm, biceps by 5.49 mm and forearm by 2.36 mm) and trunk skinfolds (suprailiac by 4.36 mm and subscapular by 2.51 mm) were thicker than in the controls ($P<0.05$). The arm-to-leg and trunk-to-arm skinfolds' ratios confirmed predisposition to accumulate adipose tissue in the upper part of the body in women with PCOS.
Fasting glycaemia and glycaemia 2 hours after of 75 g glucose intake, triglycerides and total cholesterol-to-HDL ratio was higher, but HDL was lower in PCOS women than in the controls ($P<0.05$). Fasting insulin level, HOMA-IR, total cholesterol, LDL and CRP level in PCOS and control women were similar ($P>0.05$).
Conclusions
Adipose tissue in PCOS women tends to accumulate in the upper part of the body: on arms and trunk – which may be the distinguishing feature of PCOS women.

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P575

Effect of resistin on LH and FSH stimulated steroidogenesis in porcine ovarian follicles during estrous cycle

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Resistin is a recently discovered 12.5 kDa cysteine-rich secreted polypeptide first reported from rodent adipocytes and plays important role in the development of insulin resistance and obesity. Recently, many investigators have linked resistin to reproductive function. Resistin expression was observed in bovine and rat ovaries and showed that resistin modulate granulosa cells function such as steroidogenesis and proliferation, in basal state or in response to IGF-I *in vitro*. Our previous study showed that resistin was present in porcine small, medium and large follicles in prepubertal and normal estrous cycling animals. Additionally, resistin could modulate ovarian steroid synthesis by increasing androgen production. Porcine ovaries were collected from normal oestrus cycling crossbred gilts (6–8 months of age; Large White and Polish Landrace) at a local abattoir. Small (2–4 mm; SFs, $n=6$) and medium (4–6 mm; MFs, $n=6$) follicles collected at days 4–6 and 10–12 respectively. Ovarian follicles were cultured in the presence or absence of resistin (at doses 0.1, 1 and 10) and LH (100 ng/ml) or FSH (100 ng/ml) in M199 medium. After 24 h, conditioned culture media were collected for steroid hormone secretion (progesterone-P4, androstendione-A4, testosterone-T, and oestradiol-E2) by enzyme immunoassay (EIA) but ovarian follicles were homogenized to measurement expression of steroid enzymes (3β-HSD, CYP17, 17β-HSD, and CYP19) by western immunoblot. Statistical analyses were performed using GraphPad Prism 5 software. Data were analyzed using a one-way analysis of variance (ANOVA) test, followed by Tukey's honestly significant difference (HSD) test. We observed that resistin increased stimulated LH and FSH P4, and A4 secretion by up-regulating the steady state levels of CYP11A1, 3β-HSD and CYP17A1, and 17β-HSD and had no effects on E2 secretion and CYP19A1 expression in ovarian follicles. Direct effects of resistin on steroidogenesis suggest that resistin is a new regulator of ovary function in animals during estrous cycle.

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P576

Relation of liver enzymes with indices of insulin resistance and hyperandrogenism in different PCOS phenotypes

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Introduction
Insulin resistance is a common discriminator for a variety of chronic disorders including polycystic ovary syndrome (PCOS) and non-alcoholic fatty liver disease (NAFLD). It has recently been hypothesized that PCOS itself might be a risk for developing NAFLD.
Methods
We analyzed 560 women with PCOS (BMI: 31.19 ± 6.75 kg/m^2, age: 25.62 ± 6.05 years) diagnosed on the basis of ESHRE/ASRM criteria. The subjects were divided and compared according to the clinical phenotype: phenotype A: anovulation + hyperandrogenism + polycystic ovaries ($n=324$), phenotype B: anovulation + hyperandrogenism ($n=144$), phenotype C: hyperandrogenism + polycystic ovaries ($n=45$), phenotype D: anovulation + polycystic ovaries ($n=47$). Subgroups did not differ in BMI ($P=0.085$) but there was significant difference in age ($P=0.011$) so all statistical analyses were done with adjustment for age. During follicular phase of menstrual cycle fasting blood samples were collected for determination of glucose, insulin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), testosterone and SHBG. FAI and HOMA-IR were calculated using standard formula.
Results
There was no significant difference in levels of liver enzymes between different PCOS phenotypes (AST: $P=0.72$; ALT: $P=0.34$). Significant correlations existed in phenotypes A, B, and C between ALT and insulin ($r=0.35$, $P<0.001$; $r=0.32$, $P<0.001$; $r=0.34$, $P=0.02$ respectively) and ALT and HOMA-IR ($r=0.34$, $P<0.001$; $r=0.32$, $P<0.001$; $r=0.35$, $P=0.02$ respectively), but there was no correlation between these parameters in phenotype D. There were no significant correlations between liver enzymes, testosterone and SHBG. When analyzed according to FAI, in PCOS subgroup with FAI$\geqq$6 significant correlations were found between ALT and insulin ($r=0.39$, $P<0.001$) and ALT and HOMA-IR ($r=0.38$, $P<0.001$), while there were no significant correlations between those parameters in subgroup with FAI$<$6.
Conclusion
PCOS phenotypes had different influence towards development of NAFLD. Besides insulin resistance, it seems that androgens may additionally influence development of liver disease.

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P577

The hinge region of the lutropin receptor mediates different activation mechanisms: CG induces trans- and LH only cis-initialization

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The lutropin receptor (LHR) is associated with reproduction and becomes activated by choriogonadotropin (CG) or lutropin (LH) resulting in different physiological functions with regard to differing signaling cascades. The underlying mechanisms in receptor/hormone interaction are not yet understood. CG is known to induce trans-activation at LHR oligomers (activates a second receptor), but details of LH mechanisms were not clarified yet.
To study this issue, we here used the LHR variant lacking exon 10 (hLHR-delEx10) encoding 27 residues of the extracellular hinge region, because it was previously reported that in contrast to CG, LH-induced cAMP accumulation is decreased at hLHR-delEx10. We, therefore, hypothesised a potential relation between the hinge region and differentially initialized signal induction by the hormone subtypes.
Since coexpression of binding- and signaling-deficient LHR mutants can restore CG-induced function, we assumed that CG maintains full signaling capacity at hLHR-delEx10 by trans-activation, while the lack of exon 10 might disturb trans-activation of hLH. Thus, we coexpressed the hLHR-delEx10 and the hLHR Lys605Glu mutant – in which signaling is abolished – with the binding-deficient mutant hLHR-C131R. In contrast to CG, LH only activates LHR via cis- (bound protomer), but not via trans-activation.
Structure predictions and advanced structural models suggest that exon 10 encoded and consecutive residues likely form two helical elements. In accordance to this, block-wise poly-alanine (structure preserving) mutations within this region showed no effect on signaling. Surprisingly, the structure disturbing double-proline mutant LHR-303P/305P within exon 10 has – in contrast to hLHR-delExon10 – no impact on hLH, but on hCG signaling, while a single proline mutation after exon 10 showed wild type-like functions.
In conclusion, our complementary structural and functional insights support that the structure of exon 10 encoded and proximate residues of LHR function as spacer elements interacting differently with LH and CG by mediating distinct cis- and trans- initialization of hLHR signaling dependent on the hormone subtype.

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P578

***AMHR2* polymorphism and risk for polycystic ovary syndrome: relationship to gonadotropin levels**

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Introduction
The polycystic ovary syndrome (PCOS) is a common and complex disease without a clear pattern of inheritance. There is evidence that PCOS is affected by both genetic and environmental factors, but there is no single and unambiguous explanation for its pathogenesis. Anti-Müllerian-inhibiting hormone (AMH) has an inhibitory effect on FSH-stimulated follicle growth and it has also been shown that serum AMH levels are higher in women with PCOS than in normovulatory women. The elevated AMH levels may reflect abnormalities at the AMH receptor. The current study examined the AMH receptor 2 (AMHR2) −482 A>G polymorphism (rs2002555) in a large cohort of PCOS women. The large number of the participants as well as their ethnic homogeneity enhanced the ability to detect a potential correlation between this polymorphism and the PCOS syndrome.
Methods/design
In 858 Caucasian women with PCOS and 312 healthy controls, hormonal determinations and *AMHR2* −*482 A>G* polymorphism genotyping were performed.
Results
The *AMHR2* −*482 A>G* gene polymorphism (rs2002555) was more common in women with PCOS than in controls ($P=0.026$). The relationship between *AMHR2* −*482 A>G* and PCOS remained when examining only subjects meeting NIH criteria for PCOS. Homozygous *AMHR2* −*482 A>G* gene polymorphisms (GG) were associated with decreased levels of LH ($P=0.003$) and LH:FSH ratio ($P=0.01$) in women with PCOS, as well as with lower prolactin levels ($P=0.004$). No other associations related to *AMHR2* −*482 A>G* polymorphisms were observed in women with PCOS or controls.
Conclusion
In this study, the role of the *AMHR2* −482 A>G gene polymorphism in the pathogenesis of PCOS has been suggested by the association of the variant with PCOS risk. The role of *AMHR2* −482 A>G SNP in PCOS might result from the diminished improper AMH signaling implicated in altered ovarian function.

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P579

White blood cells levels and PCOS: direct and indirect relationship with insulin resistance, but not with hyperandogenemia

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Background
Polycystic ovary syndrome (PCOS) is a complex metabolic disorder and hyperandrogenism and insulin resistance constitute the key players of syndrome pathogenesis. Another emerging important factor of PCOS is low-grade chronic inflammation. The aim of the present study was to investigate white blood cells count (WBC) in PCOS and assess its role as a possible marker of low-grade inflammation.
Methods
Anthropometrical, metabolic and hormonal data were analyzed from 236 women with PCOS (Rotterdam criteria)
Results
In the total population studied, white blood count was significantly correlated with BMI ($P<0.001$), testosterone, free testosterone, SHBG ($P<0.001$), HOMA score ($P=0.001$), E2, FG score, HDL ($P<0.001$), TGL ($P<0.001$) and insulin ($P<0.001$). Partial correlation analysis, controlling for BMI and waist:hip ratio, revealed statistically significant correlation of WBC with SHBG, TGL and HDL. In addition, multiple linear regression analysis showed that BMI and SHBG were the main predictors of white cell count in PCOS. Finally, grouping the whole sample based on the number of the white cells, we observed that the biggest rate of women with >6.000/μl WCC has BMI>27 kg/m^2, independently of the presence of normal or abnormal hormonal profile.
Conclusions
Chronic low-grade inflammation and increased white cell count do occur in PCOS. The most important factors that contribute to this inflammatory state are insulin resistance and obesity, whereas hyperandrogenism does not seem to affect it.

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P580

Interactions among resistin and peroxisome proliferator activated receptor-γ (PPAR-γ) in porcine ovarian follicles.

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Resistin, a new 12.5 kDa cysteine-rich protein, which is specifically secreted by adipocytes and describe as a potential link between obesity and insulin resistance. Recently, resistin and PPARs receptors was found in reproductive tissue such as ovary and it was found that resistin modulate ovarian follicle function. Additionally, thiazolidinediones, such as rosiglitazone, a synthetic agonist of

PPAR-γ down-regulated resistin expression in rodents. The aim of the study was to analyze i) basal resistin and PPAR gene and protein expression and ii) effect of resistin and rosiglitazone on PPAR-γ gene and protein expression. Porcine ovaries were collected from normal oestrus cycling crossbred gilts (6–8 months of age; Large White and Polish Landrace) at a local abattoir. Small (2–4 mm; SFs, $n=6$), medium (4–6 mm; MFs, $n=6$) and large (8–12 mm; LFs, $n=6$) follicles collected at days 4–6, 10–12, and 16–18 respectively. Ovarian follicles were cultured in the presence or absence of resistin (at doses 0.1, 1 and 10 ng/ml) or rosiglitazone (at doses 25 and 50 μM, diluted in DMSO) in M199 medium. After 24 h, conditioned culture media were removed but ovarian follicles were homogenized to measurement expression of PPAR-γ receptor by immunoblot and real-time PCR in all follicles. Additionally, basal resistin and PPAR gene and protein expression were also determined. Statistical analyses were performed using GraphPad Prism 5 software. Data were analyzed using a one-way analysis of variance (ANOVA) test, followed by Tukey's honestly significant difference (HSD) test. We demonstrated that basal PPAR was increased with maximum expression in LFs but resistin expression was unchanged in ovarian follicles. Moreover, both resistin and rosiglitazone increased PPAR-γ expression. In conclusion, our study provides the novel evidence interaction among resistin and PPAR-γ in the pig ovary and suggest involvement of this receptor in the control of key ovary function.

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P581

Differences in adrenocortical steroid response to ACTH in women with post-adolescent severe acne and PCOS

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Introduction
Increased adrenocortical production appears to be associated with acne and hirsutism in PCOS. However, the etiological role of androgens in the pathogenesis of acne *per se* is far from being clear. In the present study, we aimed to evaluate adrenocortical function in women with post-adolescent severe acne in comparison with patients with PCOS and healthy women.
Design
The study included 32 women with post-adolescent severe acne, 32 women with PCOS and 32 age and BMI-matched healthy controls (age 17–34 years, BMI: 20.8 ± 1.9 kg/m^2). Women with acne did not have hirsutism/biochemical hyperandrogenism or ovulatory dysfunction whereas all PCOS patients had androgen excess and ovulatory dysfunction. Measurements included basal testosterone (T), SHBG and DHEAS levels and serum 17-hydroxprogesterone (17-OHP), androstenedione (A4), DHEA and cortisol levels in response to ACTH stimulation.
Results
T, FAI, DHEAS levels, basal and AUC (area under the curve) values for A4 were significantly higher in PCOS than women with acne and controls ($P<0.05$ for all) whereas three groups did not differ for basal or AUC values of DHEA and cortisol. Women with PCOS and severe acne had significantly and similarly higher AUC values of 17-OHP compared to controls ($P<0.05$).
Conclusion
Women with isolated post-adolescent severe acne do not have increased levels of adrenal androgens basally or in response to ACTH. However, these women have similar secretion pattern of 17-OHP with PCOS patients suggesting increased enzymatic activity in this pathway.

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P582

Ovarian function is associated with obesity in very long-term female survivors of childhood cancer

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Introduction
Obesity and gonadal dysfunction are major side effects of treatment in adult childhood cancer survivors. In the general population, obesity has a negative influence on female fertility. The aim of the study was to evaluate whether obesity and serum insulin are associated with decreased ovarian reserve markers in childhood cancer survivors.
Methods
We performed a retrospective single-center cohort study in 191 adult female survivors of childhood cancer. Median age at follow-up was 27.1 (range 17.7–50.0) years and median follow-up time was 18.8 (2.3–48.8) years. Outcome measures were serum levels of anti-Müllerian hormone (AMH) and total follicle count (FC) and – if measured during early follicular phase or amenorrhoea – antral follicle count (AFC). Potential risk factors were body mass index (BMI), body composition measures, determined by dual energy X-ray absorptiometry (total fat percentage, lean body mass and visceral fat percentage) and fasting insulin. Multiple linear regression analysis, adjusted for potential confounders, was used to evaluate the associations between potential risk factors and serum AMH and FC.
Results
Lower serum AMH was found in obese subjects (β (%) −49, $P=0.007$), and in subjects with fasting insulin in the highest tertile (β (%) −43, $P=0.039$). Total fat percentage tends to be associated with serum AMH (β (%) −2.1, $P=0.06$). Survivors in the highest tertile of insulin had significant lower FC than survivors in the lowest tertile (β −6.3, $P=0.013$). BMI and other measures of body composition were not associated with FC. Correlation between serum AMH and AFC was $\rho=0.32$ ($P=0.08$).
Conclusions
Obesity and insulin resistance are associated with gonadal damage, as reflected by decreased AMH and reduced FC in adult survivors of childhood cancer. In contrast to its highly predictive value for AFC in the healthy female population, serum AMH does not seem to correlate as well with AFC in childhood cancer survivors.

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P583

New endocrine and intracellular regulators of ovarian functions

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This is the review of original data concerning the role of some metabolic hormones (GH, leptin, ghrelin, obestatin), growth factors (IGF-I, IGFBPs, EGF, thrombopoietin), intracellular mediators of their action (cyclic nucleotides, protein kinases, PKA, MAPK, CDK, transcription factors, CREB, STAT-1, p53 and related cDNA, siRNA and miRNA gene constructs) on basic ovarian functions (cell proliferation, apoptosis, secretion, oogenesis, ovulation, production and viability of pups) in different species (pig, rabbit, humans and chicken). These hormonal and intracellular regulators are able to control apoptosis, proliferation and secretory activity in porcine, rabbit, human and chicken ovarian cells and maturation of porcine oocytes and cumulus oophorus *in vivo* and *in vitro*, as well as to suppress or promote the response of ovarian cells to other hormones. Immuno-blockade of these hormones prevented their effects. Effects of hormones on ovarian cells were associated with changes in protein kinases and transcription factors in such cells, whilst blockers of kinases prevented or promoted hormones action. Transfection of granulosa cells with gene constructs for some transcription factors affected ovarian cell functions and modify hormones action. Down-regulation of approximately 1/3 known protein kinases by specific siRNA constructs resulted decrease in accumulation of these kinases within granulosa cells and changes in expression of kinase-dependent transcription factors, markers of cell proliferation, apoptosis and release of steroid hormones and IGF-I. Transfection of granulosa cells with constructs up and down regulating expression of some miRNAs are able to alter ovarian cell proliferation, apoptosis, as well as the hormone release. *In vivo* experiments demonstrated that leptin, IGF-I, steroid hormones and some regulators of PKA, MAPK and CDK could be used to predict reproductive efficiency, for direct *in vitro* control of maturation of oocytes and for *in vivo* stimulation of reproduction. Therefore, metabolic hormones, growth factors and intracellular regulators and mediators of their action (protein kinases, transcription factors, siRNAs, miRNAs) can be used for characterization of state of ovarian cells, for identification signaling pathways controlling reproductive processes, as well as for prediction and control of basic ovarian cell functions.

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P584

Deletions of *TCF2* gene in Rokitansky syndrome (MRKH): a new candidate gene? About two new cases

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Introduction
MRKH syndrome is a rare congenital disease, which affects 1/5000 female births. It is usually diagnosed in the course of primary amenorrhea investigation. Characteristics are mullerian agenesis with 46XX karyotype. Only *Wnt4* gene (1) was involved in a few cases of MRKH with hyperandrogenism. We describe two new cases with MRKH syndrome and complete deletion of *TCF2* gene in the heterozygous state, this gene is also involved in monogenic diabetes type 5.
Case 1
Seventeen year old, primary amenorrhea, karyotype 46XX, normal puberty. Clinical examination: 3 cm vagina, MRI: absence of uterus, normal ovaries. Ultrasound: 7 mm unsignificant kidney cyst. Biological results: normal plasmatic levels of creatinine, liver enzymes and fasting blood glucose (0.72 g/l).
Case 2
Six years old left kidney atrophia, 14 years insulinated diabetes, diagnosis of primary amenorrhea at 17 years old, karyotype: 46XX, normal puberty. At clinical examination: utero-vaginal aplasia. Occurrence of renal deficiency and increase level of liver enzymes during follow up.
Molecular biology
Complete deletion of *TCF2* gene in the heterozygous state in these two cases.
Conclusion
TCF2 gene is involved in MODY 5 diabetes, encoding HNF1B a nuclear transcriptional factor expressed in several organs (liver, lung, pancreas, kidney and genital tract). 18% of women with TCF2 mutations have kidney and genital tract abnormalities (bicornuate uterus). To date only three cases have been reported, two siblings in 1999 (4) and a 24 weeks fetus in 2008 (5). It was a partial *TCF2* deletion in heterozygous state in these three MRKH syndrome. With this two new cases associating MRKH syndrome and complete *TCF2* deletions in the heterozygous state, with an unsignificant renal cyst in one case, we suggest that this gene should be tested, regarding it's implication during the follow up (renal failure, diabetes, liver enzymes).
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P585

Thrombin generation in polycystic ovary syndrome: a marker of thrombosis

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Background
Polycystic ovary syndrome (PCOS) is clinically associated with increased risk of CVD. Thrombin generation (TG) is a measure of the thrombotic potential. This can be expressed as endogenous thrombin potential (ETP) representing the total amount of generated active thrombin over the time (nmol/l×min). ETP is considered the most predictive parameter of thrombosis and is associated to both venous and arterial thrombotic disease. Setting: PCOS women prospectively referred to three Danish Gynecological clinics due to infertility, hirsutism and oligo-/amenorrhea.
Materials and methods
One hundred and forty-eight PCOS women diagnosed according to the Rotterdam criteria, mean age 27 years (range 18–40). Measurements: BMI, plasma TG, hsCRP, PAI-1, HOMA-IR, body composition (DXA) and urinary-albumin/creatinin. The calibrated automated thrombogram was used to measure TG.
Results
ETP levels were divided into tertiles:
Total and free testosteron, u-albumin/creatinin did not correlate to ETP. The correlation to total fat mass (TFM) seemed equal to both android and gynoid fat. ETP did not correlate to number or combinations of the Rotterdam criteria. In a univariate analysis, ETP correlated to the majority of the above-mentioned parameters. In a multivariate analysis including TFM, HOMA-IR, hsCRP, total and LDL-cholesterol, triglycerides, ETP was independently associated with TFM and total cholesterol ($\beta = 0.358$, $P = 0.025$; $\beta = 0.482$, $P = 0.022$ respectively).

Table 1

	1134.5 ≤ 1695	1695.1 – 1913.5	1913.6 – 3026	*P*-value (ANOVA)
n	50	49	49	
BMI	25±4	27±4	28±4	0.004
HOMA-IR	0.84±2.6	0.98±2.1	1.24±3.2	0.040
Total fat mass (kg)	7.7±2.6	9.7±2.8	10.3±3.2	0.001
hsCRP	0.57±2.9	0.97±3.3	1.41±2.8	0.000
PAI-1	11.6±4.6	13.9±1.9	17.6±1.9	0.015

Conclusion
Increasing ETP is associated with a number of parameters traditionally associated with increased risk of CVD in PCOS. This association seems mainly driven by total FM and cholesterol in PCOS. The Rotterdam criteria were unable to detect this signal of potentially increased risk of thrombosis.

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P586

The combination of genetic variants in the FSHR and FSHB genes affect serum FSH in women of reproductive age

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Introduction
The relationship between SNPs of the FSHR gene and serum FSH has not been completely clarified. Genetic variants of the FSHB gene have been associated to variation in gene transcription and to serum FSH levels in men. An interesting joint effect of both FSHB −211G>T and FSHR 2039 A>G on male reproductive parameters has been recently observed. No data have been published on the effect of the FSHB −211G>T in combination with the FSHR 2039 A>G in women.
Description of methods
To investigate the effect of *FSHB −211G>T* together with the *FSHR 2039 A>G* on serum FSH in women we conducted a prospective study including 193 healthy eumenorrhoic women of reproductive age. In all women, early follicular phase FSH and AMH were measured by commercial assays and antral follicle count was measured by transvaginal ultrasound. Genomic DNA was purified from total peripheral blood and genotyping for the two SNPs was performed by HRM technique.
Results
No significant gradients of increasing or decreasing day 3 FSH across the *FSHR 2039* (AA/AG/GG) and *FSHB −211* (GG/GT/TT) genotypes, respectively, were observed. When women were stratified according to the *FSHR 2039* and the *FSHB −211* genotypes a statistically significant reduction of day 3 FSH was shown in the group of women with the *FSHB −211 GT+TT/FSHR2039 AA* genotype compared to the *FSHB −211 GG/FSHR2039 GG* genotype.
Conclusion
The reduction of day 3 FSH evidenced in the group of women with the *FSHB −211 GT+TT/FSHR2039 AA* genotype confirms a possible additive effect of the different SNPs in *FSHR* and *FSHB* on regulating serum FSH in women.

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P587

The hypothalamo–pituitary–adrenal axis sensitivity in women with polycystic ovary syndrome

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Introduction
It has been shown that most women with polycystic ovary syndrome (PCOS) have increased adrenal androgen production, enhanced peripheral metabolism of cortisol and elevated in urinary excretion of its metabolites. Simultaneously, this increased cortisol clearance in PCOS was shown to be followed by a compensatory overdrive of the hypothalamo–pituitary–adrenal (HPA) axis. The aim of this study was to determine HPA axis sensitivity in women woth PCOS.
Methods
We studied 65 non-obese women with PCOS (group PCOS; 24.7±4.0 years; 22.5±3.5 kg/m^2) aged 20 years and BMI matched healthy women (group controls; 27.3±5.3years; 21.9±1.9 kg/m^2). PCOS was diagnozed using ESHRE/ASRM criteria. In all subjects during follicular faze of menstrual cycle levels of cortisol, ACTH, DHEAS, testosterone, androstenedione and 17-OH-progesterone were determined. Overnight dexamethasone test with 0.5 mg was performed with subsequent determination of morning cortisol.
Results
PCOS and controls significantly differed in SHBG (40.9±22.9 vs 65.9 ±31.2 nmol/l, $P=0.021$) and FAI (9.1±8.1 vs 3.6±1.4%, $P=0.018$), while DHEAS, 17-OH-progesterone, androstenedione, basal cortisol, ACTH did not differe between groups. PCOS supressed cortisol less than controls after 0.5 mg of dexamethasone (85.0±16.9% vs 93.3±3.2%, $P=0.001$).
Conclusion
Lower HPA axis sensitivity women with PCOS with simultaneously normal basal cortisol and ACTH levels could be a mechanism for compensatory overdrive of HPA axis in this patients.

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P588

Value of lipid accumulation product for the assessment of metabolic syndrome in women with polycystic ovary syndrome

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Introduction
Women with polycystic ovary syndrome (PCOS) have higher prevalence of metabolic syndrome (MS) than healthy, age and BMI matched women. The aim of this study was to determine if novel abdominal adiposity index – lipid accumulation product (LAP), could predict MS in women with PCOS.
Methods
PCOS was diagnosed using ESHRE/ASRM criteria. We evaluated 218 non-obese PCOS women (PCOS group: 22.4±3.6 kg/m^2; 24.8±4.6 years) and 44 non-obese, BMI-matched healthy women (control group: 21.3±3.2 kg/m^2; 28.4±4.9 years). MS was diagnosed according to JIS criteria (waist circumference cut off 80 cm) in 29/218 (13%) PCOS women, while no woman in controls group had MS. PCOS group was divided into subgroups: PCOS with MS (27.3±2.8 kg/m^2; 27.4±6.2 years) and PCOS without MS (21.6±3.0 kg/m^2; 24.3±4.2 years). In all subjects blood pressure (BP) and waist circumference (WC) were determined. Blood samples were collected in follicular phase of menstrual cycle for determination of basal glucose, insulin, HDL-cholesterol, triglycerides, testosterone, SHBG and DHEAS. Systolic and diastolic BP (SBP and DBP, respectively) were determined. LAP was calculated using formula ((WC-58)×triglycerides). Homeostatic model (HOMA) index and free androgen index (FAI) were also calculated using standard formula.
Results
There was no difference in LAP between PCOS and controls (20.4±19.3 vs 18.2±23.5, $P=0.27$). PCOS without MS had significantly lower LAP than PCOS with MS (15.4±12.4 vs 51.6±26.2, $P<0.001$) and there was no difference in LAP between PCOS without MS and controls ($P=0.56$). In PCOS group LAP correlated with HOMA ($r=0.29$, $P<0.001$), FAI ($r=0.40$, $P<0.001$), SBP ($r=0.26$, $P<0.001$), DBP ($r=0.23$, $P<0.001$). JIS criteria for MS, FAI and LAP entered binary logistic regression analysis which showed that independent predictors for MS in PCOS group were LAP ($P=0.002$), SBP ($P=0.001$) and HDL-cholesterol ($P=0.001$).
Conclusion
LAP is useful surrogate marker for the assessment of MS in women with PCOS.

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P589

The assessment of metabolic derangements including metabolic syndrome in relation to the degree of hyperandrogenism in women with polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is associated with a higher risk for development of metabolic syndrome (MS). In this study, we evaluated the degree of metabolic disorders in women with PCOS and different degree of hyperandrogenism (HA).
Methods
We analyzed 234 women with PCOS (group PCOS; BMI: 22.4±3.6 kg/m^2, age: 24.8±4.7 years) diagnosed on the basis of ESHRE/ASRM criteria, and 45 healthy BMI-matched women who comprised the control group (group controls; BMI: 21.3±3.2 kg/m^2, age: 28.3±4.9 years). Women with PCOS were divided into three subgroups according to the presence of HA: i) with biochemical HA ($n=123$), ii) with clinical HA ($n=61$), and iii) without clinical or biochemical HA ($n=50$). In all subjects, basal blood samples were collected in follicular phase of menstrual cycle for determination of glucose, insulin, total cholesterol (TC), HDL, LDL, triglycerides, apolipoproteins A1, A2, B and E, lipoprotein(a), C-reactive protein (CRP) and uric acid. HOMA index was calculated using standard formula, and lipid ratios TC/HDL, LDL/HDL, triglycerides/HDL, ApoB/ApoA1 were determined. MS was diagnosed according to JIS criteria.
Results
In comparison to PCOS subgroup B and C, PCOS subgroup A had higher triglycerides ($P<0.001$ and $P=0.012$) and CRP ($P=0.032$ and $P=0.022$), lower ApoA1 ($P=0.045$ and $P=0.007$), and higher TC:HDL ratio ($P=0.02$ and $P=0.007$) and triglycerides:HDL ratio ($P<0.001$ and $P=0.001$), respectively. PCOS subgroup A in comparison to PCOS subgroup C had significantly lower HDL ($P<0.001$) and higher ApoB:ApoA1 ratio ($P=0.045$). There was higher prevalence of MS in PCOS subgroup A (21%) in comparison to groups B (3%) and C (3%), $P<0.001$.
Conclusion
PCOS women with biochemical HA had unfavorable lipid profile and higher prevalence of MS than PCOS women without hyperandrogenemia.

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P590

Gynecological aspects of prolactinoma

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Prolactinoma is a pituitary adenoma producing prolactin. Central hypogonadism is a major consequence of prolactinoma leading, in women, to menstrual troubles

and infertility. The aim of this study was to specify gynecological characteristics of prolactinomas.
Patients
This retrospective study included 28 female patients with prolactinoma. Mean age at diagnosis was 35.46 years (ext: 20–78). Mean serum prolactin level was 3442.4 ng/ml (ext: 31.9–76 915). Mean size of adenoma was 17.26 mm (ext: 5–60). It was a microadenoma (<1 cm) in 32.1% ($n=9$) and a macroadenoma in 67.9% ($n=19$) of cases.
Results
At diagnosis, history taking reveled a pregnancies average of 1.67 (ext: 0–8) for spontaneous pregnancies and 0.13 (ext: 0–2) for induced ones. Abortions average was 0.30 (ext: 0–2). Parity average was 1.24 (ext: 0–5). Among patients, 78.9% were not using any contraception. Intrauterine device (IUD) was used in 15.8% of patients and progestin in 5.3% of them. Diagnostic circumstances were galactorrhea in 15% ($n=3$), amenorrhea-galactorrhea in 35% ($n=7$), menstrual disturbance in 30% ($n=6$) and tumoral syndrome in 20% of cases ($n=4$). All patients were treated with dopamine agonists. No patient was treated by surgery. After diagnosis, hypogonadotropic hypogonadism was persistent in 20% of patients ($n=4$). Fifty per cent of women were using IUD as contraception, 33.3% had no contraception and 16.7% was on progestin. Thirteen pregnancies occurred in seven patients (four microadenomas and three macroadenomas). The number of pregnancies by patient was 1, 2 and 3 in respectively 21.4% ($n=3$), 14.3% ($n=2$) and 14.3% ($n=2$) of patients. During pregnancy, 66.7% of women ($n=4$) used dopamine agonists with a favorable outcome. Two of them had a macroadenoma.
Conclusion
These results highlight the huge gynecological impact of prolactinoma. A delayed diagnosis or insufficient treatment of this affection may lead to infertility or complicated pregnancies.

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P591

Levonorgestrel inhibits human endometrial cell proliferation through the up-regulation of gap junctional intercellular communication via the increased expression of Connexin43 and the nuclear translocation of its Ser255 phosphorylation

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Context
Gap junction intercellular communication (GJIC) and its constructed protein Connexin (Cx) participates in cell apoptosis and tumorigenesis. Our clinical studies demonstrated that a levonorgestrel (LNG)-releasing intrauterine system can reverse atypical endometrial hyperplasia.
Objective
This study assesses whether LNG exerts anti-proliferation effects on human endometrial cells through changes in the GJIC function and Cx43 expression.
Methods
The cell proliferation and apoptosis of human endometrial stromal cells (HESCs) and glandular cells (HEGCs) treated with LNG in a dose- and time-dependent manner. GJIC change and further total along with serine 368 and 255 phosphorylated Cx43 were measured.
Results
In all, 5×10^{-5} mol/l LNG revealed a time-dependent inhibition of cell proliferation and an increase of apoptosis in both HESCs and HEGCs. Furthermore, these cells demonstrated a significant GJIC enhancement upon treatment with 5×10^{-5} mol/l for 48 h. The effects of LNG were most noticeable in HESCs rather than in HEGCs. Associated with these changes, LNG induced a relative increase in total Cx43 in a time-dependent manner but not Ser368 phosphorylated Cx43, which was measured in HESCs using western blot analysis. Furthermore, laser scanning confocal microscope confirmed the increased expression of total Cx43 in the cytoplasm and, interestingly, detected the nuclear translocation of Ser255 phosphorylated Cx43.
Conclusions
LNG likely inhibits the proliferation and promotes apoptosis in HESCs and HEGCs though an increase in gap junction permeability *in vitro*, which is achieved through the upregulation of Cx43 expression and the translocation of serine 255 phosphorylated Cx43 from the plasma to the nuclear compartment.

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P592

Overnight 1 mg dexamethasone androgen suppression test is useful diagnostic tool in hyperandrogenism.

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Introduction
Low dose dexamethasone (DEX) androgen suppression test (LDDAST) is considered a tool to distinguish between the sources of androgen excess and to exclude/confirm autonomy of androgen overproduction.
Aim
To assess whether 1 mg DST can be used instead of LDDAST.
Materials and methods
Thirty-three consecutive women with hyperandrogenism age 18–38 years undergone overnight 1 mg DEX androgen suppression test and LDDAST. Testosterone, androstendione, dehydroepiandrosteron sulfate were measured initially, after 1 mg DEX and after 2 days of 2 mg DEX. 50% of initial level was considered the cut off of suppression.
Results
All but three patients (91%) who achieved less than 50% suppression of testosterone after 1 mg DEX did not suppress testosterone after 2 mg. All who suppressed after 1 mg also suppressed after 2 mg. 100% women did not achieve the cut off of DHEAS suppression after 1 mg, but all achieved after 2 mg of DEX. All but three patients (91%) who suppressed androstendione of less than 50% after 1 DEX did not suppress after 2 mg. The percent of suppression was however borderline after 1 and 2 mg. All who suppressed after 1 mg also suppressed after 2 mg. Three patients without 50% suppression of testosterone after 1 mg DEX achieved the cut off after 1 and 2 mg of androstendione. Three patients who did not achieve the cut off in androstendione after 1 mg but did after 2 mg did not suppress testosterone after 1 and 2 mg and all had PCOS diagnosed.
Conclusions
1 mg DAST with assessment of testosterone and androstendion can be used in outpatient fashion instead of 2 mg LDDST. Suppression of androstendione after 1 mg DEX is complementary in case of lack of testosterone suppression after 1 mg DEX but presence after 2 mg.

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P593

Low dose oral contraceptive use and the risk of thrombosis in polycystic ovary syndrome

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Background and aim
Oral contraceptives (OC) are the mainstay of long-term treatment for women with polycystic ovary syndrome (PCOS). OC use is associated with increased risk of venous thromboembolism. The aim of the present study was to determine whether treatment with a low dose OC induce changes on coagulation and fibrinolysis in women with PCOS.
Materials and methods
Sixteen lean, normal-glucose tolerant patients with PCOS (mean age, 22.4 ± 2.4 years; mean BMI, 20.0 ± 2.5 kg/m^2) and aged 16 years and BMI-matched healthy control women were included. All participants were non-smokers. At baseline, D-dimer, fibrinogen, activated partial thromboplastin time (APTT) and prothrombin time (PT) along with clinical, hormonal and biochemical measurements were performed in patients and controls. The studies were repeated in women with PCOS after 6 months of treatment with 0.03 mg ethinyl estradiol/3 mg drospirenone (EE-DRSP).
Results
All coagulation and fibrinolysis parameters were comparable between patients and controls at baseline. In women with PCOS; D-dimer, fibrinogen, APTT, and PT did not show a significant change after treatment ($P=0.16$, $P=0.12$, $P=0.81$ and $P=0.56$ respectively). EE-DRSP treatment resulted in a significant improvement in clinical/biochemical hyperandrogenism.
Conclusion
Our results suggest that 6 months use of EE-DRSP does not alter coagulation/fibrinolysis measured by clinical assays in lean women with PCOS.

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P594

PCOS subphenotypes stratification reveals an apparent increase of metabolic syndrome frequency in patients with concomitant hyperandrogenism and menstrual irregularities

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Background
PCOS-associated increased metabolic risk may be selective, owing to its heterogeneous nature. Identification of high-risk individuals could enable better studies and prevention of complications.
Aim
To characterize the metabolic risk, as metabolic syndrome (MetS), of specific PCOS subphenotypes.
Subjects and methods
Romanian PCOS (Rotterdam criteria) and 64 controls, 18–35 years. PCOS subphenotypes were defined: i) oligo-amenorrhoea (OA), hyperandrogenism (H), and polycystic ovarian morphology (P), OA–H–P ($n=143$, 59.58%); ii) OA–H ($n=35$, 14.83%); iii) H–P ($n=17$, 7.08%) and iv) OA–P ($n=45$, 18.75%). MetS was defined by harmonized IDF (2009) criteria. Total testosterone (TT), free-androgen index (FAI), insulin-sensitivity index (QUICKI) and SHBG are expressed as mean ± SEM and compared by ANCOVA, adjusting for BMI and age. MetS prevalences were compared by χ^2-test.
Results
MetS prevalence was higher in OA–H–P and OA–H (28.24 and 35.48%) than in controls (10.42%) ($P<0.01$). MetS frequencies (13.33 and 17.07%) of H–P and OA–P were not significantly different than controls or OA–H–P and OA–H.
The OA–H–P and OA–H had higher TT (0.84 ± 0.02 and 0.89 ± 0.07 vs 0.67 ± 0.07 ng/ml, $P<0.05$) and FAI (9.59 ± 0.98 and 7.78 ± 1.04 vs 2.88 ± 0.42, $P<0.01$) and were more insulin-resistant (QUICKI = 0.321 ± 0.003 and 0.313 ± 0.007 vs 0.352 ± 0.005, $P<0.05$) than controls.
Insulin-sensitivity did not differ significantly between the H–P and OA–P and controls (0.318 ± 0.013 and 0.321 ± 0.006 vs 0.352 ± 0.005). SHBG was lower in OA–H–P and OA–H (58.15 ± 5.34 and 61.49 ± 10.42 vs 132.71 ± 19.39 nmol/l, $P<0.01$) than in controls.
Conclusions
The oligo-anovulatory hyperandrogenic PCOS have the highest MetS prevalences, suggesting they represent more severe PCOS. The ovulatory and normoandrogenic PCOS subphenotypes were not different than controls in MetS prevalence and insulin-sensitivity, suggesting less metabolic risk. However, direct comparison did not identify significant differences of the same parameters between these two and the more severe PCOS subphenotypes.

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P595

Association of serum 25-hydroxyvitamin D and glucose levels in polycystic ovary syndrome

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Background
Women with polycystic ovary syndrome (PCOS) frequently suffer from metabolic disturbances, in particular from pre-diabetes and diabetes. Conflicting results currently exists on the relationship between vitamin D and glucose metabolism. Hence, the aim of our study was to investigate the association of 25-hydroxyvitamin D (25(OH)D) levels and glucose tolerance in PCOS women.
Methods
Cross-sectional study including 23 PCOS patients (mean age 27 years). 25(OH)D levels were measured by chemiluminiscence (Cobas e 601 by ROCHE). Standard 75 g oral glucose tolerance test were performed. Results were analysed by SPSS 18.0.
Results
Serum 25(OH)D concentrations was 18.8 ± 7.5 ng/ml. The prevalence of 25(OH)D insufficient (<30 ng/ml) and deficiency (<10 ng/ml) was 88 and 16% respectively. Three (12%), four (16%) and 16 (64%) women were patients with diabetes, glucose intolerance and normoglycemia respectively. PCOS women with glucose disturbance had lower 25(OH)D levels than PCOS women with normoglycemia (13.7 ± 7.5 ng/ml vs 21.03 ± 6.6 ng/ml $P<0,05$). In binary logistic regression analyses, 25(OH)D (OR 0.84, $P<0.05$) was independent predictors of glucose metabolic alteration in PCOS women. We found significantly negative correlations of 25(OH)D level with basal glucose (r −0.44, $P<0,05$).
Conclusion
Our results suggest that low 25(OH)D levels are associated with diabetes and glucose intolerance in PCOS women. Large intervention trials are warranted to evaluate the effect of vitamin D supplementation on glucose metabolic disturbances. PCOS women evaluate the effect of vitamin D supplementation on glucose metabolic disturbance in PCOS women. Glucose metabolic disturbances in PCOS women.

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P596

Prevalence of prediabetes state is not equal in all phenotypes of polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) frequently accompanied by insulin resistance metabolic abnormality. There are limited data on metabolic complications in women belonging to the PCOS phenotypes as defined by the Rotterdam criteria. Our objective was to investigate the prevalence of impaired fasting glucose (IFG) metabolism and dyslipidemia between different phenotypes in Iranian infertile women with PCOS.
Methods
We conducted a cross sectional study in our infertility outpatients clinic (Royan Institute), Tehran, Iran. A total of 633 women with PCOS according to Rotterdam criteria were studied (February 2011–September 2012). All subjects underwent blood samples for fasting glucose, lipids, reproductive hormones and a transvaginal ultrasound. Subjects were divided into following four different phenotypes: A) oligomenorrhea + hyperandrogenism + PCO, B) oligomenorrhea + hyperandrogenism, C) hyperandrogenism + PCO, and D) oligomenorrhea + PCO. Variables were compared between these phenotypes.
Results
The mean age was 28.7 ± 4.5 years, mean duration of infertility was 7.4 ± 4.4 years, 1.3% were illiterate, mean BMI was 26.7 ± 3.7, 13.1% had IFG and 71.7% had high-density lipoprotein (HDL) cholesterol <50 mg/dl. Prevalence of IFG in different phenotypes of PCOS were 11.9, 33.3, 13.5 and 8.6% in A, B, C and D phenotypes respectively ($P=0.041$). 75.6, 77.8, 70.3 and 67.0% in A, B, C and D phenotypes respectively had HDL cholesterol <50 mg/d ($P=0.01$). There was no statistically significant differences in other lipid profiles between different phenotypes of PCOS patients.
Conclusions
Results of this study shows the prediabetes state and one the important cardiovascular risk factors – low HDL cholesterol – are more prevalent in B phenotype of PCOS. Classification of the metabolic complications for each phenotype will provide a guide for screening of metabolic risks of PCOS and may help to optimal treatment of these complications.

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P597

Metabolic features in patients with polycystic ovary syndrome (PCOS) during different stages of reproductive life

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Polycystic ovary syndrome (PCOS) is an important metabolic and reproductive disorder which confers substantially increased risk for type two diabetes and metabolic syndrome (MS). Our aim is to characterize the metabolic profile, insulin sensitivity (IS) and insulin secretion in control (Cw) and PCOS women

(PCOSw) during different stages of reproductive life (S1: 18–35 years old, S2: 36–44 years old, S3: 45–55 years old). A total of 131 Cw and 129 PCOSw were included, distributed as follows: S1: 69 Cw and 78 PCOSw, S2: 46 Cw and 40 PCOSw, S3: 16 Cw and 11 PCOSw. Clinical and anthropometric characteristics were evaluated. In both groups, a 2 h, 75 g OGTT was performed with measurement of glucose, insulin and lipids. HOMA-IR, insulin sensitivity index (ISI) composite, HOMAbeta, insulinogenic index and area under the curve for glucose and insulin were calculated. MS was evaluated according to the modified NCEP ATP III criteria. Chronological age, BMI, anthropometrics and fasting glucose were similar between Cw and PCOSw in the three study periods; 2-h glucose increased with age in PCOSw. Stage 1 and 2 PCOSw showed a higher prevalence of hypertension, hypercholesterolemia and hypertriglycerides compared to CW.

Table 1

	S1		S2		S3	
	Cw	PCOSw	Cw	PCOSw	Cw	PCOSw
ISI composite	6.4 (1.2–23)	4.4* (0.4–11)	6.6 (1.4–21)	5.0* (0.8–12)	5.3 (1.6–8)	4.6 (0.7–4.7)
Homa-B	383 (60–4420)	327 (60–1226)	230(41–1079)	180 (26–484)	183 (76–377)	176 (48–321)
Insulinogenic index	2.6 (0.1–26.6)	2.8 (0.1–12.6)	1.3 (0.1–6.6)	1.9 (0.1–6.5)	1.6 (0.3–5.1)	1.9 (0.5–6.6)
AUC insulin	7060 (1627–26269)	11383 * (2289–40673)	6303 (975–35881)	9331* (1809–33392)	6134 (3263–14730)	7769 (1637–31880)
MS (%)	13	25*	18	34*	13	18

The present study shows that during reproductive age, PCOSw are more affected metabolically than Cw. Nevertheless during the perimenopausal period, Cw are metabolically more perturbed showing a similar metabolic profile to PCOSw.

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P598

Association of thyroid autoimmunity and thyroid dysfunction with obstetric antiphospholipid syndrome

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Background
The association of antiphospholipid syndrome (APS) with various endocrine disorders is already known. However, data on the influence of thyroid autoantibodies and/or disorders of thyroid function on the expression of classical APS manifestations are rather limited.
Objectives
The aim of this investigation was to evaluate the impact of thyroid autoimmunity and dysfunction on frequency of recurrent miscarriages in patients with primary antiphospholipid syndrome (pAPS).
Methods
The study included 62 female patients with pAPS (mean age 43.32 ± 11.10 years), classified according to the category of antiphospholipid antibodies (aPL) into four groups: I (with more than one aPL) – 41 patients (66%); IIa (isolated presence of lupus anticoagulant) – five patients (8%); IIb (isolated presence of anticardiolipin antibodies) – 12 patients (19.5%) and IIc (isolated presence of anti-beta two GPI antibodies) – four patients (6.5%). All patients were screened for the presence of thyroid autoimmunity (anti-thyroglobulin antibody (TgAb), anti-thyroid peroxidase antibody (TPOAb) and thyroid receptor antibody (TRAb)) and thyroid dysfunction (thyroid stimulating hormone (TSH) and free thyroxine (fT4)). Data on recurrent miscarriages were obtained from the patients' medical records.
Results
Recurrent miscarriages were documented in 68% of the category I patients, all patients in the category IIa, 67% of the category IIb patients and 50% of those from the category IIc. Thyroid autoimmunity was significantly more prevalent among patients with documented recurrent miscarriages (16/44, 36%), compared with patients with no such data (4/18, 22%) ($P<0.01$). Among patients with history of recurrent miscarriages, ten patients (23%) had overt hypothyroidism, requiring levo-thyroxine replacement, while none of patients without previous miscarriages had any disorder of thyroid function.
Conclusion
Well-known association between autoimmune disorders, in the case of APS and thyroid autoimmunity, opens controversial issue of possibility of their synergistic, additive or potentiating effects on pregnancy complications as one of the classic manifestations of these relatively common syndromes.

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P599

Inflammatory markers in polycystic ovarian syndrome and their association with cardiovascular risk factors

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Objectives
i) To determine and compare inflammatory markers including adiponectin, visfatin and IL-18 in patients with polycystic ovarian syndrome (PCOS). ii) To find out whether adiponectin and interleukin-18 (IL-18) is associated with markers of insulin resistance, hyperandrogenism and carotid intima-media wall thickness (CIMT) as a cardiovascular risk factor.
Methods
This is a prospective controlled study involving 60 consecutive euglycemic patients with PCOS (Rotterdam criteria) aged 50 years and body mass index (BMI) matched controls were included in the study. After detailed clinical evaluation including anthropometry, besides oral glucose tolerance test, fasting venous samples were analysed for IL-18, visfatin, adiponectin, highly sensitive C reactive protein (hsCRP) and complete lipid profile. We estimated body composition (total body fat and visceral adipocity index, VAI by dual energy xray absorptiometry), CIMT (by Doppler ultrasonography), indices of insulin sensitivity (QUICKI) and resistance (homeostasis model assessment for insulin resistance, HOMA-IR) and free androgen index (FAI). Data were analyzed using online graphpad quickcalc software and $P<0.05$ was considered statistically significant.
Results
PCOS patients had greater FAI (1.42 ± 0.83 vs 0.64 ± 0.4), higher HOMA-IR (2.13 ± 1.05 vs 1.91 ± 1.8) and lesser QUICKI (0.156 ± 0.025 vs 0.163 ± 0.015) than the control groups. Patients with PCOS have significantly increased serum IL-18 and visfatin levels than that of the control group (IL-18: 213.48 ± 76.84 vs 170.4 ± 41.11 pg/ml, visfatin: 73.35 ± 11.54 vs 55.56 ± 9.27 ng/ml, $P<0.05$) and hsCRP (2.56 ± 0.64 vs 1.62 ± 0.78 mg/l, $P=0.004$). Similarly, the PCOS group had significantly lower level of adiponectin (0.8 ± 0.6 vs 1.04 ± 0.49 ng/ml, $P<0.001$). Correlation coefficients of IL-18 were as follows: with CIMT (0.355), FAI (0.328), HOMA-IR (0.345) and waist circumference (0.367), each with $P<0.05$. Similarly, the correlation coefficients of adiponectin were with CIMT (−0.312), FAI (−0.343), HOMA-IR (−0.352) and waist circumference (−0.359), each with $P<0.05$.
Discussion
There is alteration of adipokines and other inflammatory markers in PCOS with increase in visfatin, IL-18 and hsCRP and reduction in of adiponectin levels. Increased IL-18 and decreased adiponectin levels correlated with insulin resistance, obesity and hyperandrogenism.
Conclusion
These altered adipokine profile is associated with increased CVD risk in PCOS patients, leading to the suggestion that one of these markers like IL-18 can serve as potential therapeutic target in future for decreasing their CV risk.

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P600

The effect of metformin on pregnancy outcome among filipino women with polycystic ovary syndrome

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Background
Polycystic ovary syndrome (PCOS) affects 5–10% of women in the reproductive age group. It is associated with insulin resistance and hyperinsulinemia which is further aggravated during pregnancy. The use of metformin in PCOS is increasingly accepted but its therapeutic use during pregnancy is still a debatable

issue. There are few local studies on the use of metformin among PCOS patients and data are lacking among Filipinos.
Objectives
To determine the effect of metformin on maternal and neonatal outcome among pregnant Filipino women with PCOS.
Design
Cohort study
•**Setting:** Review of outpatient medical records at a private infertility clinic
•Population of interest: pregnant PCOS patients conceived with metformin
•Pregnancy outcome measures: Maternal outcome – rate of first trimester spontaneous abortion, development of gestational diabetes, pregnancy-induced hypertension, mode of delivery and gestational age of delivery. Neonatal outcome – live birth rates, APGAR score, infants birth weight and development of congenital anomaly.
Results
Pregnant women who continued metformin during pregnancy had lower rate of first trimester spontaneous abortion (5 vs 36.2%, P value <0.001). A nonsignificant decrease in gestational diabetes was observed among PCOS women who continued metformin throughout pregnancy (19.0 vs 32.6%, P value 0.07). No pregnancy-induced hypertension was found between the two groups. There were no differences with regard to modes of delivery and gestational age of delivery ((NSVD 44.6 vs 47.4%; CS 55.4 vs 52.6%, P value 0.77) (preterm 9.8 vs 2.7%; term 89.1 vs 97.3%; post-term 1.1 vs 0, P value 0.317)). Using multiple logistic regression, only metformin during pregnancy was significantly related with spontaneous abortion (OR = 0.168, 95% CI 0.048–0.592, P value 0.005). The differences in neonatal outcomes between the two groups did not achieve statistical significance.
Conclusion
In women with PCOS, continuous use of metformin during pregnancy reduced the rate of first trimester spontaneous abortion.

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P601

Flutamide-induced alterations in CYP17A1 gene expression and local testosterone synthesis in porcine luteal tissue – a new insight into androgens action during pregnancy

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It was established that androgens are able to modulate luteal function during pregnancy by stimulation of corpus luteum (CL) progesterone release. In pigs, CL is the main source of progesterone through the entire gestation. Our previous study revealed that porcine CL is capable of androgen synthesis during second half of pregnancy. Therefore, we suggest that androgens, in addition to progesterone, are essential for the maintenance of pregnancy in pigs.
The aim of the study was to determine whether experimentally restricted access of androgens by antiandrogen flutamide during mid- and late pregnancy influences the androgens action in porcine CL. The specific aims are i) analysis of cytochrome P450 17α-hydroxylase/c17–20 lyase (CYP17A1) mRNA expression (real-time PCR), ii) CYP17A1 protein localization (immunohistochemistry), iii) analysis of testosterone (T) production (radioimmunoassay).
Pregnant gilts were allotted into three experimental (flutamide-treated) and respective control groups. Flutamide was administered subcutaneously (50 mg/kg bw) between days 43–49, 83–89 or days 101–107 of gestation. CLs were obtained on day 50 (GD50), 90 (GD90) or 108 (GD108) of gestation, from ovaries of both flutamide-exposed and control pigs.
Overall, the administration of flutamide led to an increased T concentration in CLs obtained on GD50 and GD108, but a decreased T content in CLs on GD90. These results followed by changes in CYP17A1 mRNA expression, which was upregulated by flutamide on GD50 and GD108, but downregulated in luteal tissue on GD90. CYP17A1 was immunolocalized only in small luteal cells obtained on GD50, GD90 and GD108 from both flutamide-exposed and control animals. However, after flutamide administration, the intensity of immunostaining was higher in CLs on GD50 and GD108, but lower in CLs obtained on GD90.
In conclusion, androgen deficiency during pregnancy in pigs affects luteal T synthesis due to changes in CYP17A1 gene expression depending on the day of pregnancy.
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P602

Relation of adiponectin and leptin to antropometric and metabolic parameteres in women with polycystic ovaries syndrome

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Introduction
Women with polycystic ovary syndrome (PCOS) are characterized with insulin resistance and hyperinsulinaemia. Adiponectin and leptin are adipose tissue-specific products and are correlated with insulin resistance. The aim of the study was to access the relation of adiponectin with anthropometric, metabolic and hormonal parameters in a group of women with PCOS.
Methods
We studied 57 non-obese women with PCOS diagnosed using ESHRE/ASRM criteria (PCOS: age 24.8 ± 5.6 years, BMI: 22.6 ± 3.8 kg/m^2) and 22 BMI-matched healthy women (controls: age 27.8 ± 4.9 years, BMI: 21.8 ± 3.3 kg/m^2). Whole body fat mass (WBFM) and abdominal fat mass (AFM) were determined by bioelectric impedance (Tanita). In all subjects serum concentrations of glucose, insulin, total cholesterol (TC), HDL, LDL, triglycerides, adiponectin, leptin, testosterone, and SHBG were determined. HOMA was calculated using standard formula.
Results
PCOS had more WBFM in comparison to controls (18.47 ± 7.68 kg vs 13.02 ± 5.83 kg, $P = 0.004$), while there was no difference in AFM between groups. PCOS had higher testosterone (2.6 ± 1.0 vs 1.7 ± 1.0 nmol/l; $P < 0.001$) and lower SHBG (42.3 ± 26.1 vs 64.3 ± 29.9 nmol/l; $P < 0.001$). There were no differences between PCOS and controls in adiponectin (8.4 ± 3.5 vs 9.8 ± 3.8 μg/ml; $P = 0.15$), leptin (19.1 ± 17.9 vs 16.4 ± 16.1 μg/ml; $P = 0.47$), and HOMA index (3.3 ± 1.8 vs 3.2 ± 1.7; $P = 0.76$). There were no differences between groups in other measured parameters. Positive correlation in both groups was found between adiponectin and HDL (PCOS: $r = 0.46$, $P < 0.001$; controls: $r = 0.53$, $P = 0.01$), and leptin with WBFM (PCOS: $r = 0.71$, $P < 0.001$; controls: $r = 0.68$, $P = 0.001$), AFM (PCOS: $r = 0.63$, $P < 0.001$; controls: $r = 0.73$, $P = 0.001$) and HOMA (PCOS: $r = 0.41$, $P = 0.002$; controls: $r = 0.52$, $P = 0.016$). Leptin had negative correlation with SHBG only in PCOS group ($r = -0.42$, $P = 0.001$). Other measured parameters did not correlate with leptin and adiponectin in both groups.
Conclusion
We did not show difference in plasma adiponectin and leptin concentrations between our group of PCOS women and BMI matched healthy controls. Leptin showed better correlation with obesity and insulin resistance in comparison to adiponectin in both groups. Analyses on larger number of subjects for the relation of adipokines with metabolic indices in PCOS is needed.

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P603

PON1-108 TT and PON1-192 RR genotypes are more frequently encountered in Greek PCOS than non-PCOS women, and are associated with hyperandrogenemia

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Objective
To investigate the frequencies of three paraoxonase (PON)1 polymorphisms in Greek polycystic ovary syndrome (PCOS) and non-PCOS women, and their genotypes in association with hyperandrogenemia and insulin resistance.
Design
Case–control genetic association study.
Patients
PCOS cases (NIH criteria) and 112 controls.
Main outcome measure
Genotyping of the c.−108C > T (PON1-108), the c.163T > A (PON1-55) and the c.575A > G (PON1-192) polymorphisms and measurement of baseline androgen and insulin resistance profile.

Results
The PON1-108 TT and PON1-192 RR genotypes were more frequently encountered in the PCOS than in the control group. The PON1-192 R allele frequency was greater in the PCOS than in the control group. Comparing the PCOS and the control groups, statistical significances favored a recessive and a dominant genetic model, respectively, for the single PON1-108 T and PON1-192 R alleles. Free androgen index (FAI) levels were higher in patients with PON1-108 TT, while testosterone, FAI and dehydroepiandrosterone sulfate (DHEAS) levels were higher in patients with PON1-192 RR than in patients with the wild or the heterozygous genotype.
Conclusions
The decreased PON1 activity-associated PON1-108 TT and the PON1-192 RR genotypes are more frequently found in Greek PCOS women and are associated with hyperandrogenemia. Hyperandrogenemia must depend also on other genetic factors because the same genotypes were not associated with hyperandrogenemia in the control group. Through identification of the involved polymorphisms women with PCOS could potentially have a better therapeutic screening.

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P604

C-reactive protein and gestational diabetes mellitus

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Aim
The aim of this study was to investigate the role of C-reactive protein (CRP) in gestational diabetes mellitus.
Material and methods
We studied 65 healthy pregnant women aged 29.75 ± 5.59 years. All women referred for a 100–g oral glucose tolerance test following an abnormal result on a screening. The demographic data, waist circumference, height, and weight of the participants were recorded. Fasting levels of insulin, triglycerides (TG), CRP, fasting blood glucose (FBG) and HbA1c were measured.
Results
Based on oral glucose tolerance testing, participants were divided into two groups: normal glucose tolerance (NGT) ($n=17$) and gestational diabetes mellitus (GDM) ($n=48$). The mean CRP level was highest in GDM group (9.67 ± 5.57 mg/l), followed by NGT (2.56 ± 1.73 mg/l), ($P<0.0001$). The mean FBG (4.98 ± 0.51 vs 4.48 ± 0.58 mmol/l, $P<0.05$), HOMA-IR (3.14 ± 1.63 vs 1.83 ± 0.62, $P<0.05$) and TG levels (1.95 ± 0.65 vs 1.35 ± 0.58 mmol/l, $P<0.05$) in the women with GDM were significantly higher than those in the NGT group.
CRP was positively correlated with first trimester pregnancy BMI ($r=0.51$, $P<0.05$), HOMA-IR ($r=0.47$, $P<0.05$), HbA1c ($r=0.44$, $P<0.05$), HOMA-IR ($r=0.47$, $P<0.05$) and FBG ($r=0.41$, $P<0.01$).
By multivariate logistic regression analysis, we showed elevated CRP levels to be independent risk factors for the development of hyperglycemia.
Conclusions
In women with GDM, there is evidence of increased CRP during the first trimester. The further evaluation will be needed to clarify this association.
The further evaluation will be needed to clarify this association.

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P605

Ovarian functions and polycystic ovary syndrome in women with type 1 diabetes mellitus

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Background
Type 1 DM has been shown to be associated with increased rate of hyperandrogenemia, polycystic ovarian changes (PCO) and PCOS. Although stimulated 17-OH progesterone responses to leuprolide has previously been shown to be increased in pubertal girls with type 1 DM, the effect of gonadotropin releasing hormone analog stimulation has not been studied in adult women.
Materials and methods
Fifty-two adult women with type 1 DM and 15 healthy adult women were included in the study. Participants were evaluated with basal total and free testosterone, androstenedione, DHEAS, FSH, LH, estradiol, PRL and TSH levels. Buserelin test was performed to all participants and FSH, LH, estradiol and 17-OH progesterone responses were measured six hourly intervals for 24 hours. Patients were evaluated in means of menstrual function, ovulation, clinical or laboratory hyperandrogenism and PCO on ovarian USG.
Results
The mean age and body mass index (BMI) of the patients and the control group were similar. Patients with type 1 DM had higher free testosterone and 17-OH progesterone levels. Basal and stimulated FSH and LH responses to buserelin were found to be similar in both groups, but although non-significant, estradiol and 17-OH progesterone responses were found to be mildly elevated in patients with type 1 DM compared to control. PCOS was detected in 34.6%, hyperandrogenemia in 44.2%, clinical findings of hyperandrogenism in 28.8%, PCO on USG in 24.5% and menstrual irregularity in 36.6% of the patients. BMI, diabetes duration, daily requirement of insulin and HbA1C did not show statistically significant differences in subgroups of type 1 DM patients with or without PCOS.
Conclusion
Type 1 DM is associated with increased basal free testosterone and 17-OH progesterone levels. FSH and LH responses to buserelin is preserved, but estradiol and 17-OH progesterone responses to buserelin are mildly increased in women with type 1 DM.

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P606

Concordance study of pathological results of fertility hormones

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Introduction
During a normal menstrual cycle, serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone (P4) can vary widely between cycles for the same woman, as well as between different women. Reliable reference values based on the local population are important for correct interpretation of laboratory results.
Aims
To evaluate the discrepancy of the results on fertility hormones by two different techniques.
Material and methods
We analyzed 130 patient samples processed: ci16200 ARCHITECT (Abbott) by chemiluminescent microparticle immunoassay (CMIA), and COBAS E-411 (Roche) by electrochemiluminescence immunoassay (ECLIA). We compared normal and pathological findings for women in follicular (FP), ovulatory (OP), luteal phase (LP), in menopause and in men, according to the normal ranges of each manufacturer. Discrepant pathological values are considered an increase/decrease in 5% of concordant results between two techniques.
Results
The results provided by COBAS increase E2 30%, FSH 17%, LH 39% and P4 just 4%. Reviewing the results of the assays performed is concluded that the pathological discrepant for low cutoff (LC) and high cutoff (HC) are present: E2, LC: 17.3% in OP, 18% in LP, −9.5% in FP, −7.9% in menopause. HC: 11.8% in FP, 10.2% in LP, −20.5% in menopause. FSH, LC: 17.7% in OP. HC: −9.2% in LP. LH, LC: −6% in FP and −16% in menopause. HC: 8.5% in men and −6.1% in FP. P4, LC: 13.4% in men and FP. HC: −57% in men, −37% in FP, −5.9% in LP and −46% in menopause.
Conclusions
Normal values offered by manufacturers are very similar. The results obtained by COBAS are higher than ARCHITECT to E2, FSH and LH. There is discrepancy for P4 and E2 as there is an increase and/or decrease very marked pathological results depending on the demographic universe although slight discrepancy also exists for FSH and LH.

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P607

Main determinants of serum uric acid concentrations in polycystic ovary syndrome patients

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Introduction
Uric acid may increase cardiovascular risk, exerting proinflammatory, pro-oxidant and proliferative actions at the endothelial cell level. Classic and non-classic cardiovascular risk markers cluster in women with polycystic ovary syndrome (PCOS), uric acid being proposed as one of them. The studies available at present regarding serum uric acid levels in PCOS patients are scarce and controversial. The aim of the study was to examine the relationship between uric acid, obesity and insulin resistance in obese and non-obese patients with PCOS.
Methods
Thirty-eight overweight and obese women with PCOS and 30 controls matched for age and BMI were included in this study. Anthropometric variables, hormonal and metabolic profiles including measurements of uric acid and insulin levels were evaluated in both groups. Insulin resistance was quantified by homeostasis model assessment (HOMA).
Results
We did not find any statistically significant differences in uric acid levels between PCOS women and controls, but obese PCOS patients had significantly higher levels of uric acid compared to overweight women with PCOS. Obese patients with PCOS also had significantly higher levels of HOMA compared with overweight women with PCOS. In the control group, even the levels of uric acid was higher in obese compared to overweight women, the difference was not statistically significant. A significant positive correlation was found between uric acid levels and BMI, waist circumference, insulin levels and HOMA in PCOS group. Using stepwise linear regression analysis, only BMI which was responsible of 42.1% of the variability observed in serum uric acid concentrations, was retained by the regression model.
Conclusions
Our results suggests that obesity is the main determinant of plasma uric acid concentrations in PCOS patients. Insulin and HOMA are also involved in a lesser extent, but their role remain to be clarified in further studies.

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P608

Vitamin D level in polycystic ovary syndrome patients is not different from healthy control in Turkish population

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Introduction
Polycystic ovary syndrome (PCOS) is widely diagnosed among young women in reproductive age. Vitamin D deficiency has been known as a common problem in all ages and also in a recent study which performed in a small study groups, reported that it may accompany with hyperandrogenism. The aim of our study is to evaluate serum $25(OH)D_3$ levels in PCOS patients compared with healthy control and relation to androgen levels.
Methods
Forty-three native patients diagnosed as PCOS according to Rotterdam criteria and 24 healthy controls were recruited to the study. Weight, length, waist circumference, fat mass, glucose, insulin, lipid profiles, total and free testosterone, DHEAS, FSH, LH and $25(OH)D_3$ levels were measured during follicular phase of menstruation. BMI and HOMA-IR were calculated.
Results
Age, BMI, waist circumferences and fat mass were not different between the groups. Vitamin D levels in PCOS patients were lower than healthy control (18.9 ± 8.2, 20.6 ± 6.6 ng/ml respectively) but the difference was not statistically significant ($P > 0.05$). When PCOS group was divided in two subgroups according to the androgen levels (21 normoandrogenic; 24 hyperandrogenic), the result was not changed. Total and free testosterone and DHEAS levels of hyperandrogenic PCOS group were insignificantly higher than control and normoandrogenic PCOS group ($P < 0.001$, $P < 0.05$ and $P < 0.001$ between groups respectively). There was no correlation between androgen and vitamin D levels.
Conclusion
Vitamin D deficiency is very common in Turkish population and we found that it is same for PCOS patients. In our study, androgen levels were not correlated with $25(OH)D_3$ levels, thus we could not have found any difference between the groups according to the vitamin D levels.

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P609

Prevalence of polycystic ovary syndrome in infertile patients in Albania

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Introduction
Polycystic ovary syndrome (PCOS) is the most common gynecological endocrinopathy. Women with PCOS are at increased risk of reproductive problems including infertility, endometrial cancer, late menopause and also metabolic aberrations, including insulin resistance, type 2 diabetes mellitus, dyslipidemia and cardiovascular diseases. There is very few data available on prevalence of PCOS on infertile population in Albania. The aim was to evaluate the prevalence of PCOS among infertile women according to Rotterdam criteria.
Methods
This is a cross sectional study which included 544 participants who presented to a infertility clinic in Tirana city from January 2010–December 2011. PCOS were diagnosed using universal assessment of ultrasonographic parameters, hormonal profiles and clinical histories. Women with other endocrinological disorders were excluded from the study.
Results
The mean age of study population was 30 ± 8 years. 155 (28.5%) women had regular menstrual cycle and no sign of hirsutism or acne. One hundred and seventy-seven women (32.5%) had menstrual symptoms such as oligo-anovulation without hirsutism or acne. Thirty-eight (6.9%) manifested androgenic symptoms with regular menstrual cycle. The prevalence of PCOS was 38.7% (95% CI 34.6–42.9%).
Conclusion
Rotterdam criteria were effective for diagnosis of PCOS.
Keywords
PCOS, Rotterdam Criteria, hirsutism, infertility.

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P610

Association of advanced glycosylation end products receptor polymorphisms with coronary heart disease in postmenopausal women

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Background
Recent studies have suggested an important role of advanced glycosylation end products receptor (RAGE) in the development of atherosclerosis. However, investigation of the relationship between RAGE polymorphisms (-429T/C, -374T/A) and coronary heart disease (CHD) has shown contradictory results. Furthermore, hyperandrogenemia in women postmenopause has been associated with an adverse CHD risk profile.
Aim of the study
To investigate any possible relationship between RAGE polymorphisms with CHD and the association of RAGE polymorphisms with cardiovascular risk factors in postmenopausal women.

Methods
Ninety-six menopausal women (28 diabetics – 68 non-diabetics, mean age: 68, 34 years) who underwent coronary angiography were genotyped for the −429T/C and −374T/A variants of RAGE. In this group, androgen and estrogen levels, lipid parameters, glucose, HbA1c and insulin were determined and free androgen index was calculated.
Results
There was no significant difference in RAGE polymorphism frequencies between women with CHD confirmed in coronary angiography and those without CHD, although a cardioprotective trend was disclosed for 374AA polymorphism. Regarding hormonal/metabolic profile, women with homozygosity for the −429 allele (TT) had significantly lower levels of HDL (43,7 ± 17.16 vs 52,95 ± 14.47, $P=0.036$) and SHBG (42.73 ± 18.17 vs 52.55 ± 20.71, $P=0.038$) compared to heterozygotic subjects (TC) and significantly higher levels of triglycerides (151.75 ± 50.65 vs 125.89 ± 36.64, $P=0.042$), FAI (1.85 ± 1.43 vs 1.17 ± 0.84, $P=0.042$) and androstenedione (1.61 ± 1.01 vs 1.22 ± 0.58, $P=0.031$). Women with homozygosity for the −374 allele (AA) had significantly lower LDL levels (83.8 ± 21.96 vs 105 ± 42.97, $P=0.046$) compared to heterozygotic (AT) subjects.
Conclusion
Our data did not demonstrate an association between polymorphisms of the RAGE gene and CHD in menopausal women. However, homozygosity for the −429 allele (TT) of RAGE is associated with an adverse lipid profile and hyperandrogenemia and its role as a predisposing factor of atherosclerosis needs further evaluation.

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P611

Association of gonadotroph pituitary macroadenoma and contiguous brain chordoma in a young woman with ovarian hyperstimulation syndrome

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Introduction
Among pituitary adenomas comprise approximately 10% of benign pituitary adenomas. These adenomas often arise in middle-aged men presenting with tumoral syndrome and endocrine profile of primary hypogonadism. Gonadotroph adenomas are more difficult to diagnose in women generally in perimenopausal or postmenopausal periods.
Case report
A 33-year-old woman with a 10 years of history of sterility with dysmenorrhea and pelvic pain in relation to recurrent ovarian cysts for 1 year. Because of gradually increasing pelvic pain associated with headache, the patient presented to our outpatient clinic for evaluation. Ultrasound examination revealed bilaterally enlarged ovaries containing multiple 2–3 cm cysts. She had no neurologic or visual complaints. An endocrine evaluation showed an FSH of 9.5 mIU/ml, LH of 0.3 mIU/ml, PRL of 680 mU/l, testosterone of 0.5 ng/ml, E2 of 1100 pg/ml, and α-subunit of1.2 ng/ml. Cerebral magnetic resonance imaging examination revealed a homogeneously enhancing mass of 21 × 22 × 18 mm within the pituitary, extending to the optic chiasm and to sphenoid and a second cystic tumor inside the prepontine cistern. The patient underwent a transphenoidal resection of both tumors. Histologic examination was consistent with a benign pituitary gonadotroph adenoma with cytoplasm immunoreacting focally with antibodies against FSH. The second tumor was a chordoma. Adjuvant radiotherapy was indicated for the residual tissue. The evolution was marked by disappearance of pelvic pain. The postoperative ultrasound examination showed normal ovaries without cysts. However, the patient remains in amenorrhea because of postoperative gonadotroph deficiency.
Conclusion
This case highlights the importance of endocrine assessment in the case of recurrent ovarian cysts. Moreover, this case arises the question of a potential link between gonadotroph tumors genesis and a contiguous tumor. Finally, we note the difficulty of management of such tumors especially after surgical treatment and radiotherapy in regard to fertility.

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P612

Menstrual function in women of reproductive age with acromegaly

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In purpose to characterize the disturbances of menstrual function in acromegalic women of reproductive age, we investigated 19 women with *de novo* acromegaly diagnosed at the age of 19–37 (median age 28) years. Before diagnosis, an average duration of acro-signs was 3.8 years, after diagnosis we observed women for 5 years. Among the first meaningful symptoms menstrual irregularities and/or infertility were noted in 13 (73.7%) cases. Initial levels of GH were 71 (31–135) mMe/l, IGF-1 775 (638–971) ng/ml, hyperprolactinemia was noticed in four (21%) cases. Macro-/microadenomas were found in 17/2 (89.5/10.5%) cases. Most of the patients ($n=12$, 63.2%) had neurosurgery, afterwards eight of them started therapy with somatostatin analogues (SSA). Seven (36.8%) patients received only medical therapy ($n=5$ SSA; $n=2$ SSA + dopamine agonists). After treatment seven (36.8%) patients had controlled acromegaly (four after surgery, three on SSA), in other patients GH and IGF-1 levels were above target criteria. Menstrual disturbances were observed in four (21.1%) cases (persisted in two patients, and a hypogonadotropic hypogonadism developed after surgical treatment in two women with initially normal menstrual cycle). In other women with initial menstrual dysfunction a restoration of the normal menstrual cycle was noted due to basal treatment of acromegaly. Three patients became pregnant during treatment with SSA (two with controlled acromegaly and one with partially controlled), one of them wished to terminate a pregnancy, two others did not stop the SSA therapy and gave birth in time to healthy babies.
Conclusions
Different menstrual cycle disorders coincided with the first symptoms of acromegaly in women of reproductive age. Algorithm of examination of women in reproductive age should include a minimum screening for acromegaly. We support the point of view that SSA therapy (in our study – Sandostatin LAR) is not harmful for a fetus and could be continued during pregnancy in women with acromegaly.

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P613

Mechanisms of menstrual disorders in type1 diabetic women

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Introduction
Type 1 diabetic women have generally more menstrual disorders than healthy women.
Objectives
Identify the main mechanisms responsible of menstrual disorders in type1 diabetic women.
Patients and methods
We conducted a comparative transversal study including 70 patients with type 1 diabetes: Thirty-three having menstrual disorders (group M) and 37 with regular menses (control group T).
We compared the clinical, metabolic and hormonal features of these two groups, in order to access a possible correlation between these parameters and menstrual disorders in these patients.
Results
Our study shows that patients who had a delayed menarche had a diabetes duration significantly longer than the other patients ($P=0.02$).
On the other hand, our patients were overdosed in basal insulin and the dose of basal insulin was significantly higher in group M (0.78 IU/kg per day vs 0.61 IU/kg per day; $P=0.008$).
Also, in group T, BMI was significantly lower in patients with 25–28 days menses compared to those who had 30–32 days menses ($P=0.02$).
Hormonal assays showed significant negative correlation between the total daily insulin dose and SHBG ($P=0,005$).
Similarly, we found that the average testosterone level increased with the insulin dose, but without any significant correlation.

Conclusion
The overdose of basal insulin may explain a large part of the menstrual disorders in type 1 diabetes. Obesity can worsen the situation and increase the menses duration.

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P614

Mitochondrial function of pregnant women with subclinical hypothyroidism and gestational hypertension

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Introduction
Subclinical hypothyroidism (subhypo) is linked to gestational hypertension (gestHT) and preeclampsia. Thyroid hormones have major influence on mitochondrial activity and the mitochondrial membrane potential (MMP) reflects the functional status of the mitochondria.
Aim
The aim was to estimate whether mitochondrial function is impaired in pregnant women with or without gestHT.
Methods
We included third trimester pregnant women consulting the department of obstetrics. The cases had subhypo (raised concentrations of TSH and normal concentrations of thyroid hormones). The second group were healthy pregnant women. The third group represented healthy non-pregnant women as controls. None of the women had any thyroid disease or medication. A blood sample was drawn to measure TSH and thyroid hormone. The mitochondrial function of stained mononuclear blood cells were measured by flow cytometry. GestHT was defined as blood pressure $\geq$140/90 mmHg.
Results
We present preliminary results of 102 patients and 40 controls. The prevalence of subhypo among pregnant women was 17%. The MMP was increased among pregnant women vs controls ($P=0.03$) and MMP was further increased among pregnant women having gestHT ($P=0.01$), an increase which was absent in pregnant women with gestHT and subhypo. Reactive oxygen species (ROS) was increased in pregnant women vs controls ($P=0.006$) but we found no differences in ROS between healthy pregnant women and women having gestHT. We have not yet examined the subgroup with subhypo.
Conclusion
The 17% prevalence of subhypo in pregnant women is higher than the commonly cited prevalence of 2–3%. The MMP of pregnant women was increased compared to non-pregnant women and further increased when gestHT was present. We suggest a possible physiological adaptation, which was absent in subhypo. This observation of an impaired mitochondrial function in subhypo may elucidate the higher frequency of complications like gestHT and preeclampsia in pregnant women with subhypo.

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P615

Hormonal disorders and breast diseases in adolescent girls

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Introduction
The breast is a hormone-dependent organ.
Purpose
To study hormonal disorders peculiarities and breast diseases in adolescent girls.
Patients and methods
Examined were 867 adolescent girls (aged 12–18), including 154 girls with breast diseases and dysmorphogenies (macromastia, hypoplasia, striae). The questioning, total clinical examination, hormonal analysis and ultrasound examination were conducted. Data are expressed as median.
Results
The investigation shows that 41 (26%) girls had mastopathy (dysplasia), 107 (12%) breast striae, four girls had macromastia, two patients had breast hypoplasia and five adolescent girls had intense permanent mastalgia.
In patients with mastopathy and macromastia estradiol level was 361.3 (136.4; 368.1) pmol/l, LH level was 6.95 (1.1; 30.8) UI/l, ?SH level was 2.57 (0.51; 7.44) mUI/l, insulin level was 26.3 (6.82; 26.8) mcUI/ml, kisspeptin level was 0.05 (0.04; 0.37) ng/ml, testosterone level was 2.0 (0.8; 11.5) pmol/l and progesterone level – 0.43 (0.1; 0.9) nmol/l. The investigation shows that girls with stria cortisol level was 440.5 (175; 589.1) nmol/l. In girls with breast hypoplasia 17-OPH level was 1.17, 0.98, 1.1, 5.6 and 3.3 ng/ml, respectively. In adolescent girls with intense permanent mastalgia prolactin level was from 1022 to 1473 mUI/l. In healthy adolescent girls estradiol level was 150.3 (47.7; 217.8) pmol/l ($P=0.01$); LH level was 4.2 (1.3; 9.3) UI/l and ?SH level – 1.5 (0.8; 2.04) mUI/l ($P=0.03$); insulin level was 9.5 (5.9; 16.1) mcUI/ml and kisspeptin level – 0.03 (0.03; 0.06) ng/ml ($P<0.01$); cortisol level was 231.6 (109.7; 371.1) nmol/l ($P=0.01$); testosterone level was 0.9 (0.07; 1.2) pmol/l; prolactin level – 245.1 (109.6; 331.4) and progesterone level was 5.23 (2.9; 38.9) nmol/l ($P<0.01$).
Conclusions
High estradiol, LH, ?SH, insulin, kisspeptin, testosterone, prolactin, 17-OPH and low progesterone level are the most specific hormonal disorders in adolescent girls with mastopathy and breast dysmorphogenies.

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P616

Menstrual cycle length is associated with metabolic syndrome in young Korean women with oligomenorrhea

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Introduction
Although menstrual irregularity, including oligomenorrhea (OM), is associated with insulin resistance and hyperandrogenism, the relationship between the severity of menstrual infrequency and clinical phenotypes in young women with OM is unclear. We evaluated whether a longer menstrual cycle length is associated with less favorable metabolic features in young women.
Methods/design
A total of 1296 young women (aged 15–39 years old) with a menstrual cycle length of greater than 40 days and 1634 regular cycling women voluntarily participated. Metabolic parameters, insulin sensitivity index, and testosterone levels were measured. Oligomenorrheic women were divided into two groups: i) severe OM (menstrual cycle length >60 days) and ii) mild OM (menstrual cycle length 40–60 days).
Results
Women with severe OM displayed higher levels of most metabolic parameters and higher testosterone levels compared to women with mild OM. Among obese subjects (BMI ≥ 25 kg/m^2), women with severe OM had significantly higher levels of systolic blood pressure, insulin, cholesterol, and testosterone and lower insulin sensitivity indices compared to women with mild OM (even if they had similar BMI and waist circumference). Non-obese subjects displayed the same results. Severe OM was associated with metabolic syndrome (odds ratio 2.2, 95% CI 1.2–4.0) after adjustment for age, BMI, family history of diabetes, insulin sensitivity index, and free testosterone levels.
Conclusion
Oligomenorrheic women with a menstrual cycle length of greater than 60 days should be more closely monitored for metabolic syndrome than women with a menstrual cycle length 40–60 days.

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P617

Obstructive sleep apnea in Bulgarian patients with polycystic ovarian syndrome

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Introduction
OSA is a rare condition in premenopausal women that do not have PCOS and is most frequently associated with obesity. On the other hand the risk in PCOS

patients is thought to be significantly higher. The **aim** of the study was to investigate the prevalence of sleep apnea in Bulgarian patients with PCOS and/or obesity.
Methods
The study included 30 women – 11 obese, 13 nonobese PCOS and six obese PCOS patients. Anthropometric measurements, biochemical and hormonal assessment were performed for every patient. Polysomnography was carried out according to standard laboratory protocol.
Results
In nonobese patients with PCOS there were no detected cases of sleep apnea. In obese women without PCOS OSA was found in 54,5% and in obese PCOS in 33,3%.There was a strong positive correlation between the age of the patients and the number of obstructive apneas and hypopneas per hour, apnea/hypopnea index and respiratory disturbance index ($r=0,63$; $r=0,55$; $r=0,68$ and $r=0,69$ respectively). An even stronger correlation was observed between BMI and the abovementioned indices ($r=0,72$; $r=0,54$; $r=0,82$ and $r=0,81$ respectively). Patients with and without OSA differed significantly in the presence of visceral obesity according to waist-to-hip ratio and waist-to-stature ratio, the rate of metabolic syndrome, fasting IRI and HOMA-index. There was no difference in testosterone levels between patients with and without OSA.
Conclusion
Nonobese PCOS patients do not have higher prevalence of OSA. The main factors for OSA presence are age and obesity and not the PCOS status.

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P618

Plasma kisspeptin levels in polycystic ovary syndrome

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Introduction
Kisspeptin is a 54-amino acid peptide, which comprise a diverse group of peptides with many different functions related to energy metabolism and reproduction, pubertal development. It activates GPR54 receptor. Subcutaneous administration of kisspeptin-54 to women with regular menstrual cycles results in an increase in LH and FSH. All these findings show that the GPR54 system may play a role in the function of the hypothalamic–pituitary–gonadal axis. PCOS is characterized by hyperandrogenism and chronic anovulation. Frequently increased LH levels accompany to PCOS. Based on these findings, we designed this study to further characterize metastin levels in womans with PCOS and controls.
Methods
Women with PCOS and 28 healthy women, recruited from the Endocrinology Clinic of Pamukkale University, were studied. The diagnosis of PCOS was based on the revised criteria of Rotterdam. Anthropometric measurements were performed. A fasting blood sample was obtained in the morning for measurement of glucose, insulin, PRL, LH, FSH, estradiol, total testosterone, sex hormone-binding globulin, dehydroepiandrosterone sulphate (DHEA-S), 17-hydroxyprogesterone and TSH. Free androgen index and HOMA-IR values are calculated. Kisspeptin levels were also measured with an ELISA method.
Results
Women with PCOS had higher FAI, FG scores and DHEA-S levels, compared to controls. BMI, HOMA-IR values, fasting plasma glucose levels were higher in the PCOS group than in the control group. There was no statistically significant difference between the groups in terms of kisspeptin and LH:FSH ratios.
Conclusion
Some studies showed increased levels of kisspeptin in PCOS. Our study did not support these findings. This may be result of the obesity and insulin resistance that may have negative impact on kisspeptin levels. Leptin deficiency or resistance may be an explanation for a linkage between obesity, insulin resistance and PCOS.

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Relation of plasma kisspeptin levels with obesity and insulin resistance in polycystic ovary syndrome

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Introduction
Majority of polycystic ovary syndrome (PCOS) women are anovulatory, overweight/obese and insulin resistant. Kisspeptins are the endogenous ligands for GPR54 highly expressed in pancreas. Some studies showed that kisspeptins may play in the regulation of islet function and they stimulate insulin secretion from mouse and human islets. Adipose tissue acts as an endocrine organ and its function may be regulated by kisspeptins. We designed this study based on that obesity, insulin resistance and reproductive function changes may be a reflection of the disruptions in kiss system.
Methods
Forty women with PCOS recruited from the Endocrinology Clinic of Pamukkale University were studied. The diagnosis of PCOS was based on the revised criteria of the European Society of Human Reproduction an Embryology American Society for Reproductive Medicine in 2003. Anthropometric measurements were performed. A fasting blood sample was obtained in the morning for measurement of glucose, insulin, PRL, LH, FSH, estradiol, total testosterone(T), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEA-S), 17-hydroxyprogesterone and TSH. Free androgen index and HOMA-IR values were calculated. Kisspeptin levels were also measured with an ELISA kit.
Results
We searched the relationship between metastin and degree of obesity and insulin resistance. Twenty-six PCOS woman were overweight/obese, the remaining 14 were normal weight. In our study, obese/overweight PCOS woman were more insulin resistant than non-obese. We found no correlation between the kisspeptin and body mass index, HOMA-IR values.
Conclusion
There are no much studies looking this relationship in the literature. In the literature, plasma kisspeptin levels were found lower in obese and insulin-resistant PCOS. This inaccordant result may be due to small sample size or sex steroid effects on kisspeptin levels.

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Potential discriminant factors for different pcos phenotypes

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Background
Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. It is a heterogeneous disorder characterized by oligo- or anovulation (ANOV), biochemical or clinical manifestations of hyperandrogenemia (HA) and polycystic ovaries (PCO). Combination of these three characteristics makes four phenotypes of PCOS: phenotype 1 (ANOV+HA+PCO), phenotype 2 (ANOV+HA), phenotype 3 (HA+PCO) and phenotype 4 (ANOV+PCO). The differences between these four phenotypes are still undefined and the subjects of study.
Methods
We evaluate 92 PCOS women using stepwise linear discriminant analysis with phenotype as dependent variable and BMI, FSH, LH, LTH, oestradiol, testosterone, progesterone, HDL-cholesterol, LDL-cholesterol, HOMA-IR and HOMA-B as independent variables.
Results
The phenotype 1 was present in 32.6%, phenotype 2 in 21%, phenotype 3 in 28% and phenotype 4 in 14.1% of cases. We found that BMI, LH, HOMA-IR and HOMA-B were discriminante factors for different phenotypes, while the other variables did not reach statistically significant difference.

	BMI	LH	HOMA-IR	HOMA-B
ANOV+PCO+HA	27.1±6.0 kg/m^2;	7.33±4.69 IU/L;	1.49±0.34;	371.8±391.7;
ANOV+HA	29.2±8.0 kg/m^2;	5.30±3.81 IU/L;	1.32±0.39;	423.1±213.1;
ANOV+PCO	22.8±4.4 kg/m^2;	11.13±6.13 IU/L;	1.18±0.28;	535.1±365.5;
PCO+HA	24.9±5.8 kg/m^2;	4.12±2.62 IU/L;	1.40±0.49;	755.6±1436.2;

Conclusions
We concluded that LH, BMI, HOMA-IR and HOMA-B could be useful as discriminant factors in different PCOS phenotypes.

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Growth hormome IGF axis – basic

P621

Delay stature and shox gene molecular abnormalities: about 4 families

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Introduction
These last years have seen emerger identification of a large number of genes responsible of bone disease. La dyschondrosteosis: Leri weill is more important to know. It include short stature due to moderate micromelia mesomelic associated with a very characteristic deformity of the wrist: the Madelung deformity. This pathology is caused by haploinsufficiency of the SHOX gene, a gene essential in regulating the growth and remodeling of skeletal. We report observations about two young patients AD and SM six and height years old who consulted for delay stature. They had mesomelic dwarfism and Madelung deformity. Hormonal balance was unremarkable. Radiography helped diagnose LERI WEILL syndrome. The family survey has identified several cases in the two families. The genetic study is underway.
Discussion and conclusion
Located in the pseudo autosomal region of the Xchromosomia PAR1, several molecular abnormalities can effect the shox gene. It may be mutation, deletion or duplication. Others conditions of growth are associated with SHOX: The LANGER dwarfism, Turner syndrome and even some cases called idiopathic growth retardation. These conditions must be recognized early to allow effective management of affected children and their families.

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P622

A comparison of the *in vitro* production of IGFs, IGFBP-4 and PAPP-A in cultures of visceral and subcutaneous adipose tissue fragments from obese subjects

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Background
Visceral adipose tissue (VAT) is less insulin sensitive and more harmful than subcutaneous adipose tissue (SAT). In adipocytes, IGF1 receptor signaling has been linked to insulin resistance, but it is unknown whether VAT and SAT also differ in production of IGFs. Therefore, we compared IGFs, IGFBP-4 and PAPP-A production in cultured fragments of VAT and SAT from obese subjects.
Methods
VAT and SAT was collected perioperatively during elective bariatric surgery from 17 women and 9 men (mean ± S.E.M.: age 38.7 ± 1.9 years, BMI 39.8 ± 0.9 kg/m^2). Culture media collected after 48 h of incubation (500 mg tissue fragments) without (baseline) and with GH (100 μg/l) were compared for concentrations of IGFs, IGFBP-4, PAPP-A and bioactive IGF1 as measured by IGF1 receptor bioassay.
Results
VAT and SAT produced similar amounts of IGF1 at baseline (mean and inter-quartiles: 0.25 (0–0.56) vs 0.06 (0–0.23) μg/l), but VAT produced more IGF1 than SAT after GH stimulation (0.58 (0.12–1.63) vs 0.30 (0.08–0.59) μg/l, $P<0.05$). IGF2 production was markedly higher in VAT than SAT (2.66 (1.47–3.84) vs 1.65 (1.09–2.21) μg/l, $P<0.05$), and in both compartments, baseline IGF2 production exceeded that of IGF1 ($P<0.0001$). VAT produced fourfold more PAPP-A than SAT (5.16 (4.20–7.00) vs 1.16 (0.96–1.56) μg/l, $P<0.05$). VAT also produced more IGFBP-4 (45.7 (24.5–69.3) vs 22.7 (18.2–37.7) μg/l, $P<0.05$). In accordance with these observations, levels of bioactive IGF1 were higher in media from VAT than SAT at baseline (0.13 (0.03–0.19) vs 0.06 (0.05–0.10), $P<0.05$) as well as after GH stimulation (0.22 (0.10–0.37) vs 0.11 (0.05–0.18) μg/l, $P<0.05$).
Conclusion
VAT produced markedly more IGF2, PAPP-A and IGFBP-4 than SAT, resulting in a higher media IGF1 bioactivity. Furthermore, VAT was more sensitive to GH than SAT. Our data suggest that PAPP-A may control IGF-action in adipose tissue, in particularly in VAT. This mechanism may have effects on local insulin sensitivity.

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Low extracellular sodium causes neuronal distress independently of reduced osmolality in an experimental model of chronic hyponatremia

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Introduction
There is evidence that chronic mild hyponatremia (serum (Na+) 130–135 mmol/l) may have clinical consequences, such as fracture occurrence and neurological symptoms including unsteadiness, falls and attention deficits. These have been traditionally associated to water movement into nervous cells, as a result of the hypotonic state. The aim of the present study was to determine whether low extracellular sodium directly exerts negative effects on human neuronal cells, independent of reduced osmolality.
Design
We exposed two neuronal cell models (i.e. SK-N-AS and SH-SY5Y neuroblastoma cell lines) to sustained low extracellular sodium, thus mimicking a condition of chronic hyponatremia, both in the presence of reduced or unaltered osmolality, obtained with the addition of appropriate amounts of mannitol.
Results
We found that very low sodium (i.e. 115 and 90 mmol/l in SK-N-AS and SH-SY5Y, respectively) significantly reduced cell viability and adhesion. However, intermediate low sodium was able to cause cell distress, as assessed by the altered expression of anti-apoptotic genes and the reduced ability to differentiate into a mature neuronal phenotype. Noteworthy, these effects were observed also in the presence of unaltered osmolality. Moreover, we performed a comprehensive micro-array analysis in cells maintained in normal sodium or in low sodium and unaltered osmolality and we found that the most altered pathway included genes involved in 'cell death and survival'. Among the 43 differentially expressed genes, the Heme oxigenase gene, which represents a transcriptional response to oxidative stress, showed the highest increase in the expression level.
Conclusions
This study demonstrates that low extracellular (Na+) directly cause detrimental effects in neuronal cells independent of reduced osmolality. These findings further support the recommendation to effectively correct hyponatremia, even when mild and chronic.

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Association between a common GH receptor polymorphism (exon 3-deletion), pre- and postnatal growth, serum insulin-like growth factor 1 and reproductive function in 838 healthy young males

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Introduction
A common genetic polymorphism in the GH receptor (GHR) is deletion of the entire exon 3 sequence (GHRd3 isoform). The GHRd3 isoform seems to be more sensitive with increased downstream GH signalling in activated exon 3 deleted receptor dimers (GHRd3/d3) compared to full length receptor dimers (GHRfl/fl). Presence of the GHRd3 isoform might be associated to pre- and postnatal growth and pubertal development in boys, although controversy exists. The aim was to investigate the influence of exon 3 GHR polymorphism on pre- and postnatal growth, insulin-like growth factor 1 (IGF1) serum levels, IGF binding protein 3 (IGFBP-3) levels, reproductive hormone levels (gonadotropins, testosterone, estradiol, sex hormone binding globulin) and semen quality (semen volume, sperm counts, sperm motility and sperm morphology).

Method/design
Population-based study consisting of 838 healthy young males. Data on prenatal growth were available in 428 individuals.
Results
Corrected for gestational age there was a trend towards higher birth weight (standard deviation score) among GHRd3/d3 homozygotes ($n=41$) compared to GHRfl/f homozygotes ($n=241$) (0.55 ± 1.6 vs 0.15 ± 1.3, $P=0.09$). Final height ($P=0.59$), IGF1 levels ($P=0.79$) and IGFBP-3 levels ($P=0.49$) were not significantly associated with GHRd3 polymorphism.
GHRd3/d3 homozygotes ($n=69$) compared to GHRfl/fl homozygotes ($n=467$) had significantly larger semen volume (3.6 (2.6–4.7) vs 3.2 (2.4–4.3) ml, $P=0.049$) and higher serum inhibin-B levels (227 (185–264) vs 208 (158–257), $P=0.052$). Levels of other reproductive hormones and other variables reflecting semen quality were not significantly associated with the GHR genotype.
Conclusions
Presence of GHRd3/d3 genotype was weakly associated with increased testicular function as evaluated by semen volume and levels of inhibin-B but did not influence other reproductive hormone levels or semen quality. Serum IGF1 and pre- and postnatal growth were not significantly influenced by GHRd3 polymorphism.

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SNPs within the GH signaling pathway are associated with the fast, but not the long term, IGF1 response to GH replacement therapy in GH deficient adults

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Introduction
GH deficiency (GHD) in adults is associated with low serum levels of insulin-like growth factor 1 (IGF1) and a deteriorated cardio-metabolic profile. GH replacement therapy (GHRT) increases serum IGF1, an important mediator of the treatment response and safety marker of dose titration. The interindividual variation in treatment response is large and most likely influenced by genetic factors.
Aim
The aim of this study was to test the hypothesis that single-nucleotide polymorphisms (SNPs) in genes within the GH signaling pathway impact on the fast (1 week) and/or long term (6 months and 1 year) IGF1 response to GHRT.
Patients and methods
Three hundred and thirteen consecutive GHD subjects (58.1% men; mean age 49.7 years) were studied before and after 1 week, 6 months and 1 year of GHRT. Response was defined as the percentage of change in IGF1 levels from baseline (fast response) and from 1 week (long term response). Six SNPs in the GHR, JAK2, STAT5b, SOCS2 and PIK3CB genes were selected for genotyping.
Results
Average genotyping success rate was 98.8%. In linear regression analyses adjusting for sex, age and the GH dose, SNPs rs6873545 (GHR) and rs361072 (PIK3CB) were significantly associated with the percentage of change in IGF1 levels at 1 week of GHRT ($P=0.031$ and $P=0.017$ respectively). No SNP was significantly associated with the long term response.
Conclusion
Our results indicate that common genetic variants in genes with function in the GH signaling pathway may be of functional relevance to the fast response to GHRT in GHD adults. In addition, these results highlight the fact that genetic factors are more likely to impact on the fast response to RT, when factors such as individual dose titrations, diet and life style choices etc. are less likely to influence the response marker.

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Pilot study of nocturnal TSH surge in idiopathic short stature

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Background
TSH surge is the most sensitive test currently available for confirming the diagnosis of central hypothyroidism, a hidden cause of idiopathic short stature.
Objective
Studying the nocturnal TSH surge in children with the idiopathic shot stature.
Patients and method
The study included 30 healthy children (10–18 years) as a control group and 30 children of matching age and sex with ISS as a patients group. TSH was measured for each subject at each of 1000, 1900, and 2200 h peak and nadir levels were determined, and TSH surge ((peak–nadir)/nadir) was expressed as percent rise over nadir.
Results
33.33% of patients with idiopathic short stature had central hypothyroidism with blunted nocturnal TSH surge. 36.67% of the patients had unrecognized mild primary hypothyroidism with normal nocturnal TSH surge. The remaining 30% rested under the cover of the term 'idiopathic' short stature and had a normal nocturnal TSH surge, thus there was a significant percentage of central hypothyroidism in idiopathic shot stature in comparison with control group who all had normal nocturnal TSH surge.
Conclusion
The study of nocturnal TSH surge in idiopathic shot stature is useful to diagnose and avoid missing central hypothyroidism and mild primary hypothyroidism.

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Preclinical characterisation of NNC0195-0092, a long-acting GH

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GH is an important regulator of longitudinal growth in children and metabolism in adults. GH is used for treatment deficiency disorders in both children and adults. GH treatment is safe and well tolerated. However, its clinical use is limited to daily s.c. injections which poses a challenge for both the patients and the physicians and has prompted research into alternative approaches to GH treatment.
NNC0195-0092 is a hGH derivative conjugated with an albumin binding moiety. The *in vivo* pharmacodynamic and pharmacokinetic properties of NNC195-0092 have been studied in hypophysectomised rats, mini-pigs and cynomolgus monkeys.
NNC0195-0092 induced dose-dependent increase in body weight of hypophysectomised rats and increase in plasma IGF1 levels in rats, mini-pigs, and cynomolgus monkeys. Multiple once weekly dosing of hypophysectomised rats induced a step-wise increase in body weight with an initial linear growth the first 3–4 days in each dosing interval. During the 28 days study period with 4 weekly doses, body weight increased by 52% compared to a vehicle control group. The IGF1 levels increased after each dose and returned to the same trough level in each dosing interval. Significant increases in body lean mass, tibia bone mineral content, and tibia cortical thickness were observed together with a decrease in body fat mass compared to a vehicle control group.
Pharmacokinetics in all three species has indicated a 1. order absorption from the subcutaneous tissue after a s.c. injection and a non-linear elimination. Apparent terminal half-lives of 5–6 h in rats, 10–12 h in mini-pigs, and 17–20 h in monkeys have been observed. The bioavailability was estimated to 39% in rats, 36% in mini-pigs, and 69% in monkeys.
In conclusion, once weekly s.c. administration of NNC0195-0092 exhibit pharmacokinetic and pharmacodynamic properties suggesting that NNC195-0092 is a potential once weekly GH candidate.

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Sheehan's syndrome females have a high incidence of cardiovascular morbidity and an increased prevalence of cardiovascular risk factors

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Introduction
While severe GH deficiency (GHD) is a well-established feature of Sheehan's syndrome (SS), the effects of GH deficiency in these patients has not been extensively investigated. In the present studyh we evaluated the cardiovascular risk in patients with SS.
Methods
Twenty female with SS and well-treated with cortisone and thyroid hormones was included. Metabolic syndrome according to IDF-2005 criteria and insulin resistance estimated by HOMA-IR were investigated. GHD was searched based on Glucagon test. Common carotid intima-media thickness (IMT) was measured by B mode ultrasound. Cardiovascular risk was assessed by SCORE.
Results
The GHD was found in 95% of patients. Hypertension represents the most frequent history of cardiovascular disease. The mean BMI was at 28.10 ± 6 kg/m^2, 35% of patients were obese. The mean waist circumference was at 99.5 ± 15 cm. Fasting glucose more than 5.5 mmol/l was present in 45% of subjects. A total cholesterol >5.2 mmol/l was observed in 65% of cases, an LDL-C >4.1 mmol/l in 35% of cases, an HDL-C <1 mmol/l in 15% and a triglyceride level >1.7 mmol/l in 60% of cases. Metabolic syndrome was present in 55% of cases. Forty percent of patients had insulin resistance. The IMT was not increased in all cases; the presence of atheromatous plaques was noted in one case. The IMT was positively correlated with diastolic blood pressure and LDL-C. Cardiovascular risk was moderate in 40% of cases and very high in 15% of cases. Patients with high cardiovascular risk had higher systolic blood pressure and fasting glucose.
Conclusion
These findings highlight the importance of closely cardiovascular and metabolic monitoring of patients with SS unsubstituted in GH and raise the question of the benefit of GH replacement in these patients.

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P629

Determinants of carotid intima media thickness in acromegaly patients

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Introduction
Acromegaly is a rare disease characterized by GH and IGF1 hypersecretion and increased cardiovascular mortality. Ultrasonographic measurement of carotid intima media thickness (CIMT) gives substantial information about early atherosclerosis. We aimed to evaluate CIMT and its determinants in a group of acromegaly patient.
Patients and methods
Forty-four acromegaly patient and 43 age, sex and body mass index (BMI) matched healthy controls were included. Blood pressure (BP), BMI, waist-to-hip ratio (WHR) measurements; plasma CRP, fibrinogen, lipid, fasting plasma glucose, GH, IGF1, insulin tests; HOMA-IR calculations and CIMT measurements were done.
Results
Right ($P=0.017$) and left ($P=0.025$) CIMT were found to be significantly higher in acromegaly group than the healthy controls. Right CIMT correlated positively with age, WHR and CRP; negatively with GH and IGF1. Besides, left CIMT showed positive correlation with age, diastolic BP, WHR, HDL-C and negative with IGF1. The lineer regression analysis demonstrated that while age and CRP was the major determinants of right CIMT, only the age was so for the left CIMT. Both right ($P=0.001$) and left ($P=0.020$) CIMT were significantly different among active acromegaly (AA), cured acromegaly (CA) and healthy control groups. Right CIMT was significantly higher in CA group than the AA group and healthy controls and left CIMT was significantly higher in CA group than the healthy controls. The higher mean of age in the CA group is thought to be a possible explanation for the increased CIMT in the CA group.
Conclusion
Studies evaluating CIMT in acromegaly patients have depicted different results so far. In this study, we found increased CIMT in acromegalics. Increased CIMT seems to be solely related to the age of the patient in paralel to previous reports. The disease activity and the other cardiovascular markers like glucose, lipids, BP are not found as confounding factors to increased CIMT.

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First results from the PATRO adults study of omnitrope for the treatment of adult patients with GH deficiency

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Introduction
More data are needed to confirm the long-term safety of recombinant human GH (rhGH) in adult patients with severe GH deficiency (GHD), particularly with regard to diabetogenic potential. PATRO Adults is an ongoing observational, multicentre, open, longitudinal study of Omnitrope, being conducted in hospitals and specialised endocrinology clinics across Europe. Here, we present safety data from an interim analysis.
Methods
Eligible patients are male or female adults who are receiving treatment with Omnitrope and who have provided informed consent. Patients treated with another rhGH product before starting Omnitrope therapy are eligible for inclusion. The overall primary objective of the study is to assess the safety and efficacy of Omnitrope in adults treated in routine clinical practice; particular emphasis will be placed on the risk of glucose intolerance or diabetes and normalisation of IGF1 levels.
Results
As of October 2012, 297 patients were enrolled in the study; 116 had received previous GH treatment. Mean (S.D.) age and body mass index of enrolled patients was 50.7 (15.4) years and 29.9 (6.8) kg/m^2 respectively. One hundred and fifteen adverse event (AEs) have been reported to date in 49 patients, with seven (in four patients) regarded as serious. Nine AEs in five patients were suspected as drug-related, with headache the most common ($n=3$). Eight patients have so far discontinued treatment (four were GHD-naive at study entry, four had received prior rhGH treatment). Of the eight patients who have discontinued, only one did so due to an AE.
Conclusions
Based on these interim results, Omnitrope treatment in adults with GHD is well tolerated in a real-life clinical practice setting, both in previously rhGH-naive and previously treated patients. The ongoing PATRO Adults study will provide important data on the diabetogenic potential and overall safety of long-term GH replacement therapy in this population.

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GH therapy in Turner syndrome: growth rate indirectly correlated with age of therapy initiation

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Introduction
Short stature is the most common physical abnormality in Turner syndrome (TS), with adult stature averaging 20 cm shorter compared to the general population.
Aim
To study the efficiency of early initiation for GH therapy on final stature for patients with Turner syndrome and to prove it's important role in obtaining optimal growth rates.

Methods
We studied a lot of 24 patients with TS diagnosed and followed up in our Department of Endocrinology. The most frequently congenital malformations associated with Turner syndrome were:
Congenital heart diseases (seven cases), Hypothyroidism (five cases) and Structural malformations of the kidneys (five cases).
Considering the diagnosis age for Turner syndrome, four categories were defined: i) 0–5 years: 5% (two patients), ii) 5–10 years: 36% (eight patients), iii) 10–15 years: 36% (eight patients), iv) 15–20 years: 23% (six patients). Recombined GH therapy was initiated for the last three categories.
Results
The growth rates were significant in the three groups:
i)age 5–10 years: average growth rate: 0.736 cm/month (18 months follow-up); average initial stature: 113.3 cm; mean total height gain:11.7 cm
ii)age 10–15 years: average growth rate:0.561 cm/month (mean follow-up 24 months); average initial stature: 124.6 cm; mean total height gain: 15.0 cm
iii)age 15–20 years: average growth rate:0.278 cm/month (mean follow-up 18 months); average initial stature 137.6 cm; mean total height gain: 4.6 cm
Conclusions
Comparing the growth curves and growth velocity in the three groups we notice that growth rates decrease with late therapy initiation, which becomes an important factor for the final stature prognosis. Longer follow-up is important for evaluating the efficiency of early initiated GH therapy.
Discussion
In patients group of 15–20 years, we obtained the lowest growth rates, the incriminated factor being the associated substitutive oestrogen therapy and it's effects on the bone plate.

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P632

Glucose metabolism abnormalities in a population of acromegalic patients

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Introduction
There is a well-established association between acromegaly and insulin resistance (IR). The abnormalities in glucose metabolism may be an important risk factor of cardiovascular morbid-mortality in these patients.
Objective
Evaluation of glucose metabolism abnormalities in a population of naïve acromegalic patients and its relationship with delay in diagnosis, gender, levels of insulin like growth factor 1 (IGF1) and GH.
Methods
Retrospective study of 98 acromegalic patients diagnosed between 1982 and 2011. Levels of glucose and insulin were evaluated during fasting and after 75 g oral glucose tolerance test. Homeostasis model assessment for insulin resistance (HOMA-IR) and for insulin secretion (HOMA-beta) was calculated using the following formulas: HOMA−IR index = (fasting insulin × fasting glucose)/22.5. HOMA-beta index = (20 × fasting insulin (mU/ml))/(fasting glucose (mMol/l) − 3.5).
Results
Seventeen patients had previous diagnosis of type 2 diabetes mellitus (T2DM), 42 had normoglycemia, five impaired fasting glucose (IFG), 12 impaired glucose tolerance (IGT), 10 IFG and IGT, four diagnosis of T2DM according to glucose fasting levels, four T2DM according to glucose levels at 120′ PTGO and four patients diagnosis of T2DM on both methods. Patients with previous T2DM were older (50.7 ± 13.2 vs 40.8 ± 14.0, $P = 0.03$) and had higher levels of HOMA-IR index (11.3 ± 11.3 vs 4.0 ± 3.5, $P = 0.005$) than normoglycemic patients. Although normoglycemic patients had higher levels of HOMA-beta index than patients on the other groups of glycemia, these differences were not statiscally significant ($P = 0.846$). There were no statistically significant differences between groups in what concerns diagnosis delay, body mass index, levels of GH and IGF1. There was a positive correlation between IGF1 levels and HOMA-IR ($r = 0.306$, $P = 0.013$).
Discussion
The prevalence of T2DM in this population is higher than in portuguese population. A considerable proportion of patients presented intermediate hyperglycemia. In this analysis, age was the only factor predisposing to glucose metabolism alterations and T2DM. There is a correlation between insulin resistance and disease activity.

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P633

Evaluation of LIAISON XL vs IMMULITE 2000 IGF1

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Introduction
Insulin-like growth factor 1 is a single-chain polypeptide of 70 amino acids. Quantitative determination of IGF1 is a one-step sandwich chemiluminescence immunoassay (CLIA). IGF1 is being used by physicians as a screening test for GH deficiency and excess. It became crucial to find an alternative automated platform for the current available methods.
Design
Evaluation took place in an HMO central lab, currently using the SIEMENS IMMULITE 2000. Seventy-nine randomly outpatients serum samples collected from Clalit Central Laboratory. Fifty-three routine samples were tested on pre-modified IMMULITE kit, delivered from Screening Department Institute of Mother and Child, Warsaw Poland. Patients, controls and NEQAS samples were tested by: DiaSorin LIAISON IGF1, IMMULITE IGF1. Results were analysed according to patients' age and gender.
Results
Correlation between LIAISON IGF1 and IMMULITE IGF1 was analysed for: A. samples from Clalit central laboratory. B. samples delivered from abroad, pre-modified IMMULITE vs LIAISON. C. Samples delivered from abroad, new modified IMMULITE vs LIAISON. D. Samples delivered from abroad, new modified IMMULITE vs pre-modified IMMULITE. The r values were 0.9750, 0.9849, 0.9750, 09758, 09770, 0.9453 and 0.9729 respectively. Total clinical agreement between new modified IMMULITE vs pre-modified IMMULITE and between pre-modified IMMULITE vs LIAISON were 85 and 100%, respectively. Correlation between LIAISON and IMMULITE IGF1 of NEQAS samples showed $r = 0.995$. Controls within run precision showed 2.36–3.35% CV. Between run precision showed 4.79–7.75% CV. Functional sensitivity showed values of 4.11 and 6.43 pg/ml. Dilution test showed linearity of $r = 0.9953$.
Conclusions
High correlation was observed between LIAISON and IMMULITE IGF1 together with high clinical agreement. It is concluded that the LIAISON IGF1 can be used as reliable and accurate kit for high throughput laboratories.

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P634

GH therapy: never say never; clinical case

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We present the case of a patient diagnosed with panhypopituitarism at an adult age. The tenth child of a poor family, PA consulted the endocrinologist accompanied by his 65 year old mother. PA was 20 years old at the first consultation. He was, however, entrapped in the body of a 12 year old child, being only 139 cm tall and having no signs of puberty onset. He was slightly eunuchoid, with long limbs, due to delayed puberty, with micropenis and lack of androgen dependent hair, but otherwise harmoniously developed. He had low blood pressure and hypoglycemia. His skin was pale, dry and slightly carotenic and infiltrated and his scalp and eyebrow hair was scarce. His clinical appearance was very suggestive for panhypopituitarism. Clinical supposition was confirmed by hormonal investigation: a low IGF1 (38 ng/ml, normal 250–600 ng/ml) and low basal GH (0.1 ng/ml, normal >10 ng/ml), which didn't increase at effort, insulin or clonidine tests, demonstrating a GH deficiency. TSH and fT_4 were below the normal limit, basal morning cortisol was low (5 μg/dl) and it maintained low values even after insulin stimulation. The tests revealed also a gonadrotopic deficiency, with prepubertal LH, FSH and testosterone. MRI investigation showed partial empty sella, ectopic neurohypophysis and hypoplastic pituitary stalk (described midline effect accompanying GH deficiency in children). After 3 years of treatment with recombinant GH, thyroid hormones and glucocorticoids, the patient gained 20 cm in height, the clinical aspect improved, as well as the paraclinical values. At the moment, he also undergoes injectable testosterone treatment thereby growing five extra centimeters (final stature 174 cm) and developing secondary sexual characteristics. This case pleads in favor of GH therapy in panhypopituitary patients irrespective of the age of diagnosis with potential beneficial effects on growth.

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P635

Silver–Russell syndrome about 15 cases and review of literature

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The Silver–Russell syndrome (SRS) is a sporadic heterogeneous disorder, clinically and genetically. The intrauterine growth retardation (IUGR), short stature because of lack of catch-up growth, relative macrocrania and limb asymmetry are the major diagnostic criterias, associated with a large clinical criterias (minor criteria).
We report a case series of 15 cases with SRS, our goal is to relate the frequency of each symptom of the diagnostic criterias and evaluate the response to a long-term treatment by the GH. The diagnosis of SRS was established by the presence of three major criterias and (at least) two minor criterias.
Indeed, all the patients showed short stature and relative macrocrania, and eight of them showed IUGR and body asymmetry. The paraclinical diagnosis (The GH deficiency) was confirmed in four of the cases. The treatment with GH whose efficacy was observed even in the absence of the deficit was reported in six cases.
Although, SRS is a rare entity, it remains under-diagnosed. In spite of the clinical score for the diagnosis – recently established – its accuracy is largely influenced by the experience of the physician. Early diagnosis with initiation of treatment with GH is primordial to improve final height in adulthood.

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P636

Neuroendocrine disorders and anomalies of median line: about two cases

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Several observations brought reported a big frequency (12–50%) of pituitary insufficiency and abnormalities of the median line. The endocrine disorders are hypothamic origin and come along of pituitary ontogeny in more 30% of the cases. These abnormalities are associated with a defect of the embryological development of the bird nasofrontal which allows the development of various regions of the encephalon and the face. We report the observations of two boys KY 18 years old and 1 year old who presented to the birth a typical syndrome malformed of the median line (labiopalatine, flate nose, cleft lip, umbilical hernia and signs of gonadotropin deficiency: micropenia with bilateral cryptorchidism. The abnormalities did not noticed by a doctor until the age of 10 and 2 years old respectively. The two boys had severe short stature (-6 SDS and -3 SDS respectively). The hormonal assessment highlighted a GH deficiency. Surgical correction was performed however the medical treatment by rGH was possible only height years later for KY and 2 years later for the other boy. The evolution is marked by the apparence of a thyreotrop insufficiency and insipid diabetes in KY. The existence of abnormalities of the median line at a child must be considered as a marker and introduce endocrine and neuroradiology investigations as well as long term follow. The appearance of the speed of growth,or signs of pituitary insufficiency need revaluation.

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Male reproduction

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***KISS1R* mutations in normosmic congenital hypogonadotropic hypogonadism: clinical evaluation of two families and molecular characterization of a novel mutation**

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Context
KISS1R mutations have been implicated in patients with normosmic congenital hypogonadotropic hypogonadism (nCHH) (OMIM #146110).
Objective
To describe in detail nCHH patients with biallelic *KISS1R* mutations belonging to two unrelated families, and to functionally characterize a novel *KISS1R* mutation.
Results
The p.Tyr313His original mutant was found in the homozygous state in three affected kindred (two females and one male) from a consanguineous Portuguese family. This mutation, located in the seventh transmembrane domain, affects a highly conserved amino acid, perturbs the conformation of the transmembrane segment, and impairs MAP kinase signaling and intracellular calcium release. In the second family, a French Caucasian male patient with nCHH was found to carry two recurrent mutations in the compound heterozygous state (p.Leu102-Pro/Stop399Arg). In this patient, pulsatile GnRH administration restored pulsatile LH secretion and testicular hormone secretion, and long-term combined gonadotropin therapy induced spermatogenesis, enabling 3 successive pregnancies that resulted in two miscarriages and the birth of a healthy boy.
Conclusion
We show that a novel loss-of-function mutation (p.Tyr313His) in the *KISS1R* gene can cause familial nCHH, revealing the crucial role of this amino acid in KISS1R function. The observed restoration of gonadotropin secretion by exogenous GnRH administration further supports, in humans, the hypothalamic origin of the gonadotropin deficiency in this genetic form of nCHH. The possible role of *KISS1R* haploinsufficiency in miscarriage is discussed.

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Strength training and testosterone treatment have opposing effects on migration inhibitor factor levels in ageing men

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Objective
The beneficial effects of testosterone treatment (TT) in men with low normal testosterone levels are debated. Chemokine levels have not previously been evaluated during TT in combination with strength training (ST).
Design
A randomized, double-blinded, placebo-controlled study of 6 months TT (gel) in 54 men aged 60–78 years with bioavailable testosterone (BioT) <7.3 nmol/l and waist circumference >94 cm randomized to TT (50–100 mg/day, $n=20$), placebo ($n=18$) or ST ($n=16$) for 24 weeks. Moreover, the ST group was randomized to TT ($n=7$) or placebo ($n=9$) after 12 weeks.
Methods
Clinical evaluation and chemokine measurements (migration inhibitor factor (MIF), monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α). Lean body mass (LBM) and regional fat mass (total, central and extremity) were established by dual x-ray absorptiometry and visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured by magnetic resonance imaging.
Results
Following 24 weeks intervention, MIF and SAT were decreased during ST+placebo vs placebo, whereas BioT and LBM were unchanged.
TT decreased fat mass (total, central, extremity, SAT) and increased BioT and LBM vs placebo. MIF levels increased during TT vs ST+placebo.
ST+TT decreased fat mass (total, central, extremity) and increased BioT and LBM vs placebo.
Following the last 12 weeks of treatment, MCP-1 levels increased during TT vs placebo and MCP-1 levels decreased during ST+placebo vs placebo.
Conclusion
ST+placebo was associated with decreased MIF levels suggesting decreased inflammatory activity. In contrast, TT may be associated with increased inflammatory activity.

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P639

Decreased expression of four memory genes in non-syndromic cryptorchid males

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Background
Egr1 has been widely recognized as essential for some aspects of learning and memory while Egr 4 has been shown to be involved in receptor – modulated memory processes. Furthermore, it has been shown that testosterone enhances memory by increasing biological salience of incoming information. In cryptorchid boys, an elevated odd ratio for low IQ was found. i) It is postulated that the hypothalamus–pituitary–testicular axis is implicated in maintaining the similarity of gene expression between brain and the testis.
Aim
We hypothesize that infertile cryptorchid males may have impaired expression of several memory genes in their testes.
Patients and methods
Whole genome analysis of testicular biopsies from seven boys who underwent orchiopexy with typical testicular histology of a high risk infertility group (HIR) were compared to 12 biopsies of cryptorchid boys with low risk for developing infertility (LIR) utilizing Affymetrix microarrays and quantitative real time PCR and immunohistology.
Results
The HIR group had low or lack of expression of the following memory genes compared to LIR: EGR1, EGR4, FMR2 and VCX3A. (Median values, log2; EGR4 2.7 vs 5.65 $P<0.0006$, EGR1 7.49 vs 9.2 $P<0.027$ FMR2 4.71 vs 6.03 $P<0.003$, VCX3A 5.9 vs 7.46 $P<0.01$) Immunohistologic analysis revealed lower EGR1/EGR4 expression in the spermatogonia of the HIR group as compared to LIR testes.
Conclusion
Impaired expression of four memory genes known to encode for proteins involved in signalling pathways regulating cytoskeleton organization, synaptic vesicle transport and establishment of connections between neuronal cells may contribute to reduced intellectual and cognitive functions of cryptorchid males. The current findings provide new insight into potential molecular mechanisms into crucial processes for the development of these functions. 1. Depue R. *Teratology* 1988 **37** 301.

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P640

Complete aromatase deficiency in four adult men: detection of a novel mutation and two known mutations in the *CYP19A1* gene

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Introduction
At present, only eight men with loss-of-function mutations in the *CYP19A1* gene have been described. Here we report the genetic study of four adult men with undetectable serum estrogens, unfused epiphyses, eunuchoid skeletal proportions, continuing linear growth, tall stature, genu valgum, osteoporosis, obesity and achantosis nigricans. Patient 1 (26-years/182 cm) and 2 (28-years/187 cm) are from Turkey with a history of consanguinity. Patient 3 (44-years/185 cm) and 4 (29-years/197 cm) are two brothers from Jordan without a history of consanguinity.
Methods
All coding exons with their flanking intronic sequences of *CYP19A1* gene, amplified by PCR, were directly sequenced by ABI-Prism 3130 Genetic Analyzer and compared with known human *CYP19A1* gene sequences.
Results
Patient 1 was homozygous for a point mutation in the first nucleotide of intron 3 (IVS3+1G>T); Patient 2 homozygous for a G>A mutation (c.1124 G>A) in exon IX resulting in protein missense mutation p.R375H. The two brothers (Patients 3 and 4) had a homozygous mutation in exon IV (c.434 G>A) leading to Arg to Gln substitution at position 115 (p.R115Q). All patients had impaired glucose tolerance, Patient 3 was diabetic, Patient 2 had a history of three forearm bone fractures after minimal trauma, Patient 1, 3, and 4 had impaired liver function and GH secretion.
Conclusions
The description of these new four aromatase-deficient men confirms the detrimental effects of congenital estrogen deficiency on glucose, liver and bone metabolism (particularly bone maturation and mineralization). The homozygous missense mutation in exon IV (p.R115Q) (Patients 3 and 4) is novel. Both aminoacids are basic, but their different conformational structure probably leads to tertiary or quaternary distortion in protein structure. The other two mutations have been previously described in heterozygosis and are found in homozygosis for the first time. Clinical evidence of documented osteoporotic fractures in an aromatase-deficient man is described for the first time.

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Mortality is increased in men treated with testosterone compared to age and sex-matched controls

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Background
Symptomatic hypogonadism is frequent and associated with increased risk of cardiovascular events. Hypogonadism is often treated with testosterone replacement. In a recent meta-analysis on adverse events in randomised placebo controlled testosterone replacement trials (RPCT), no differences between testosterone or placebo/non-intervention groups with regards to cardiovascular events or risk factors was observed. Conversely, a contemporary RPCT in elderly immobile patients was prematurely stopped due to increased rate of cardiovascular events in the actively treated patients. The cardiovascular risk in men on long-term testosterone treatment is largely unsettled.
Aim and method
We aimed at assessing the association between the exposure variable testosterone treatment and development of cardiovascular events in a cohort of all men in Denmark filing prescriptions for testosterone in the period 1997–2008. Three age and sex-matched controls per patient were randomly selected from the background population. Information on mortality, disease, drug use and social variables was acquired using four Danish nationwide registries.
Results
Seven thousand three hundred and thirty-three testosterone users were identified. Women and prior (before 1997) testosterone users were excluded, generating a study cohort of 4792 cases (aged 46.3 ± 0.3; years $\pm$ S.E.M.) and 14 376 controls (46.7 ± 0.2 years), which was followed until December 2011. In the mean observation period (13.2 ± 0.03 years) 858/1963 deaths were observed in cases/controls yielding an unadjusted total mortality odds-ratio of 1.38 (1.26–1.51, 95% CI, $P<0.01$) in cases vs controls and correspondingly a Cox proportional hazard-ratio of 1.36 (1.25–1.47, 95% CI). Unadjusted odds-ratio for cardiovascular death was 1.22 (1.04–1.43, 95% CI) in cases vs controls.
Conclusion
We found a significant 36% increased mortality in men treated with testosterone compared to age- and sex-matched controls. We also found an increased risk of cardiovascular death in the cases. However, in this on-going study additional analyses are needed to further clarify whether the observed increased mortality is connected with co-morbidity, concomitant drug.

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P642

Seminal, ultrasound and psychobiological parameters correlate with metabolic syndrome in male members of infertile couples

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Introduction
Metabolic syndrome (MetS) is a diagnostic category which identifies subjects at high risk for diabetes and cardiovascular diseases, erectile dysfunction (ED) and male hypogonadism. However, MetS impact on male infertility has been poorly studied. We systematically evaluated possible associations between MetS and clinical characteristics in men with couple infertility.
Methods
Out of 367 consecutive subjects, 351 men without genetic abnormalities were studied. MetS was defined according to the IDF and AHA/NHLBI classification. All men underwent physical, hormonal, seminal and scrotal ultrasound evaluation. Erectile and ejaculatory functions were assessed by International Index of Erectile Function-15 erectile function domain (IIEF-15-EFD) and Premature Ejaculation Diagnostic Tool (PEDT), respectively, while psychological symptoms by Middlesex Hospital Questionnaire.
Results
Out of 351 patients, 27 (7.7%) fulfilled MetS criteria. Among ultrasound features, in an age-adjusted logistic model, only testis inhomogeneity was significantly associated with increasing MetS factors (HR = 1.36 (1.09–1.70), $P < 0.01$). In an age-adjusted model, MetS was associated with a stepwise decline in total testosterone (TT) ($B = -1.25 \pm 0.33$, $P < 0.0001$), without a concomitant rise in gonadotropins. At univariate analysis, progressive motility and normal morphology were negatively related to the number of MetS components (both $P < 0.0001$), but when age and TT were introduced in a multivariate model, only sperm morphology retained a significant association ($B = -1.418 \pm 0.42$; $P = 0.001$). The risk of ED (IIEF-15-EFD score < 26) increased as a function of the number of MetS factors, even after adjusting for age and TT (HR = 1.45 (1.08–1.95), $P < 0.02$). No association between PEDT score and MetS was observed. Finally, after adjusting for age and TT, somatization and depressive symptoms were associated with increasing MetS components ($B = 0.66 \pm 0.03$, $P < 0.05$; $B = 0.69 \pm 0.03$, $P < 0.02$; respectively).
Conclusions
In men with couple infertility, MetS is associated with hypogonadism, poor sperm morphology, testis ultrasound inhomogeneity, ED, somatization and depression. Recognizing MetS could help patients to improve not only fertility but also sexual and overall health.

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Effects of iron therapy on pituitary gonadal axis and sperm parameters in adults with iron deficiency anemia (IDA)

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Objective
To evaluate semen parameters and measure serum FSH, LH, Testosterone (T) concentrations before and 6 weeks after i.v. (800–1200 mg elemental iron iron therapy in adults with iron deficiency anemia.
Patients and methods
This study investigated 11 adults with iron deficiency anemia (IDA) aged 40 ± 5 years, with full pubertal development (Tanner stage 5, eugonadal) and capacity to ejaculate. They had iron deficiency anemia mainly due to defective intake of iron. Anemia was diagnosed when Hb is = or < 10 g/dl. (Serum iron, TIBC and ferritin) concentrations confirmed the diagnosis of IDA. Basal serum concentrations of FSH, LH, and T were evaluated before and 6 weeks after i.v. iron therapy.
Results
After iv iron therapy and correction of anemia (Hb > 11 g/dl) a significant increase of hemoglobin from 8.1 ± 01.17 to 13.1 ± 0.7 g/dl was associated with increased T (from 12.22 ± 1.4 to 15.9 ± 0.96 nmol/l), FSH (from 2.82 ± 0.87 to 3.82 ± 1.08 IU/l) and LH (from 2.27 ± 0.9 to 3.82 ± 1.5 IU/l).
Total sperm count (TSC) increased significantly from 72 ± 17.5 to 158 ± 49 million/ml ($P < 0.001$), sperm volume increased from 2.3 ± 0.6 to 2.6 ± 0.7 ($P = 0.045$), rapid progressive sperm motility (RPM) increased from 22 ± 9.4 to 69 ± 30 million/ml (P, 0.001), and sperms with normal morphology (NM) increased from 33 ± 5 to 56 ± 7 million/ml ($P < 0.001$).
Hemoglobin concentrations were correlated significantly with T ($r = 0.75$, $P < 0.001$), total sperm count ($r = 0.81$, $P < 0.001$), total progressive motility ($r = 0.78$, $P < 0.001$), rapid progressive motility ($r = 0.74$, $P < 0.001$) and sperms with normal morphology (0.88, $P < 0.001$). Testosterone concentrations were correlated with TSC (0.52, $P < 0.001$), total progressive motility (0.55, $P < 0.001$) and RPM (0.55, $P < 0.001$) and NM (0.78, P 0.001).
Conclusion
We proved that correction of anemia with i.v. iron therapy is associated with significant enhancement of sperm parameters and increased concentrations of serum T, LH, and FSH. These effects on spermatogenesis are reached by an unknown mechanism and suggest a number of pathways that need further human and/or experimental studies.

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Endocrine disruptors target ATP binding cassette transporters in the blood–testis barrier and impair Leydig cell steroidogenesis

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Efflux transporters in the blood–testis barrier (BTB) prevent entry and accumulation of xenobiotics in testis but are also involved in local transport of steroid hormones. Among these are the ATP-binding cassette (ABC) transporters, *P*-glycoprotein (*P*-gp/ABCB1) and multidrug resistance proteins 1 (MRP1/ABCC1) and 4 (MRP4/ABCC4). Here, we studied the interaction of suggested endocrine disruptors (EDCs) bisphenol A (BPA), tetrabromobisphenol A (TBBPA), bis(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS) with human *P*-gp, MRP1 and MRP4-mediated transport using membrane vesicles of human embryonic kidney (HEK293) cells overexpressing these transporters. Our data show that BPA, DEHP and MEHP, did not significantly alter *P*-gp, MRP1 and/or MRP4 activity. Both PFOA and PFOS inhibited the MRPs by 50% and *P*-gp up to 25% at 100 μM. TBBPA showed complete inhibition of the three transporters at the highest concentration (100 μM) tested. To investigate the toxicological implications of transporter inhibition further, testosterone secretion and expression of steroidogenic genes were determined in mouse Leydig MA-10 cells upon exposure to EDCs. Only, BPA and TBBPA concentration-dependently induced testosterone secretion by MA-10 cells to 350 and 1000% of control at 10 μM, respectively. Blocking *P*-gp, using PSC833, further increased testosterone secretion after BPA and TBBPA exposure to 475 and 2000%, respectively. In contrast, the MRP inhibitor MK571 partly blocked testosterone secretion elicited by BPA (till 300%) and completely by TBBPA. Furthermore, expression of testis-specific steroidogenic genes StAR and CYP11A1 increased when exposed to EDCs in combination with transporter inhibitors. No effects on androgen receptor (AR) activation were found using an AR luciferase reporter assay. These data suggest that EDCs might affect male fertility by locally acting on the BTB and steroidogenesis. We propose that EDCs might disrupt local androgen production and transport from Leydig into Sertoli cells, thus potentially affecting normal germ cell development.

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Prenatal testosterone programming: ontogeny of changes in testis of fetal and prepubertal male sheep

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The reprogramming effects of an excess of testosterone during pregnancy in males born to PCOS mothers as well as in animal models for PCOS has been only recently evaluated. We have found in a sheep model of PCOS that prenatal T (PT) excess impact on the testis of adults with increased Sertoli cell (SC) number, decreased sperm count, and reduced germ cell (GC) number. The ontogeny of such disruptions and the mechanisms have not been established. This study addressed the impact of PT excess on testis histology and expression of genes

modulating testicular function in fetuses of 120 days of fetal age and in prepubertal sheep of 24 weeks of age. Males were born to mothers administered with either T propionate (T-males) or the vehicle (C-males). At both ages, histological parameters were quantified, and mRNA and protein expression of AMH and transcription factors involved in the AMH signaling as well as proteins related to the BT barrier were analyzed by RT-PCR and WB. Testicular weight was similar in fetal C-and T-males while was lower in prepubertal T-males. SC number was similar between T- and C-males fetuses, on the contrary, the SC number was increased and the number of GC was reduced in prepubertal T-males. mRNA expression of AP2 was similar in both groups and ages, but a higher expression of AMH was observed in T-males. The WB of AMH receptor (MISRII) was lower in T-males at both ages. N-cadherin protein expression was similar in T-and C-males fetuses, but lower in prepubertal T-males. Protein expression of connexin 43 was similar in fetuses, but higher in prepubertal T-males compared to C-males. These findings suggest that the altered environment impairing spermatogenesis of adult male sheep after PT exposure begins at prepubertal ages with an abnormal molecular and cellular environment.

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Androgen levels and sexual activity in young men ($n = 773$), 20–29 years of age, a population based study. Odense Androgen Study

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Introduction
Low androgen levels are associated with decreased sexual activity. However, to our knowledge no population based study has evaluated the association between androgen levels and sexual activity in young men.
Aim
To evaluate associations between androgen levels and sexual activity in young men.
Design
Seven hundred and seventy-three Danish men, aged 20–29 years, underwent clinical and biochemical examination: Total testosterone (TT), bio-available testosterone (BioT) and DHT levels) and questionnaires (sexual function). Odense Androgen Study is a population-based cohort study, the included men matched the county population of Funen (1–3).
Results
391/773 (50.6%) were living with a partner. 141/773 (18.2%) were singles with a stable sexual partner and 241/773 (31.2%) were singles without a sexual partner. 28/773 (3.6%) were virgins.
TT, BioT and DHT levels were negatively associated with time since last coitus ($r = -0.12$, $r = -0.10$, $r = -0.12$, respectively; $P < 0.001$).
Four hundred and fourteen men reported lower frequency of coitus than desired. 204/414 had a partner and 99/204 criticized low libido in their partner. These 99 men had their last coitus 5 days ago (1–28) (median (10–90 percentiles)). The number of days since last coitus was 6 days (1–60) in the remaining complainers (105/204), who were content with their partner ($P < 0.001$ vs men unsatisfied with partner). The 99 men compared to the 105 men had significantly lower TT, BioT and DHT 19.1 vs 21.7 nmol/l; 11.2 vs 12.3 nmol/l and 1.55 vs 1.71 nmol/l, respectively ($P < 0.01$).
Conclusion
3.6% of men aged 20–29 years were virgins. Androgen levels were negatively associated with time since last coitus. Men with complaints concerning partner's too low libido had fewer days since last coitus but had lower androgen levels than men who were satisfied with their partner.

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Characterization of sumoylated proteins in human sperm

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SUMOylation is a post-translational protein modification involved in the regulation of essential cell functions. Our group found several SUMO-1 and SUMO-2/3ylated proteins in human ejaculated spermatozoa (Marchiani *et al.* 2011). We showed that SUMO-1 is mainly present in live spermatozoa and the percentage of SUMOylated spermatozoa was inversely correlated with total and progressive motility. SUMOylated proteins are mainly located in the nucleus and in the midpiece. To better understand the role of SUMO modification in sperm we aimed to characterize possible target proteins. In particular, we evaluated RanGap-1 (Ran GTPase-activating protein 1) one of the main target of SUMO in somatic cells, DRP1 (Dynamin-related protein 1), whose SUMOylation in somatic cells provokes alterations of mitochondrial functions, and Topoisomerase II alpha, necessary for chromatin condensation and demonstrated to be a target of SUMOylation in germ cells.
By co-immunoprecipitation both with anti-SUMO-1 and with anti-RanGap-1 antibodies, we demonstrated that RanGap-1 is SUMO-1ylated in human sperm. With the same strategy, we showed that DRP-1 is SUMO-1ylated and that the SUMO modified protein is found at higher levels in sperm pools from asthenospermic men respect to normospermic. By confocal microscopy, we observed that the co-localization between SUMO-1 and RanGap-1 and between SUMO-1 and DRP1 are mainly found in the neck area. In addition, we also demonstrated the co-localization between SUMO-1 and Topoisomerase II in the sperm nucleus.
In conclusion, we idenfied RanGap-1, DRP-1 and Topoisomerase II as SUMOylation targets in human sperm. Our data suggest that SUMOylation could play different roles in human sperm functions and the characterization of target proteins is fundamental to reveal such roles. The higher levels of SUMOylated DRP1 in asthenospermic subjects, suggests that alterations in mitochondrial function due to SUMO-modified DRP1 may result in decreased sperm motility whereas sumoylation of RanGap may play a role in silencing gene translation.

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Characterization of R31C GNRH1 mutation in congenital hypogonadotropic hypogonadism

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Normosmic congenital hypogonadotropic hypogonadism (nCHH) is a rare reproductive disease leading to lack of puberty and infertility. Loss-of-function mutations of *GNRH1* gene are a very rare cause of autosomal recessive nCHH. R31C *GNRH1* is the only missense mutation that affects the conserved GnRH decapeptide sequence. This mutation was identified in a CpG islet in nine nCHH subjects from four unrelated families, giving evidence for a putative 'hot spot'.
Interestingly, all the nCHH patients carry this mutation in heterozygosis that strikingly contrasts with the recessive inheritance associated with frame shift and non-sense mutations.
Therefore, after exclusion of a second genetic event, a comprehensive functional characterization of the mutant R31C GnRH was undertaken. We clearly demonstrate a dramatic reduction of the mutant decapeptide capacity to bind its cognate receptor, to activate MAPK pathway and to trigger inositol phosphate accumulation and intracellular calcium mobilization. In addition it is less able than wild-type to induce *lh-beta* transcription and LH secretion in gonadotrope cells. Furthermore, the absence of a negative dominance *in vitro* offers a unique opportunity to discuss the complex *in vivo* pathophysiology of this apparently dominant form of nCHH.

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Sexual dysfunction in cirrhotic patients awaiting liver transplantation

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Introduction
Data on sexual dysfunction (SD) in cirrhotic patients are limited. Sexual function is a complex area of human behavior with great impact on quality-of-life (QOL). Despite its relevance, it is rarely evaluated in clinical practice in cirrhotic patients. Indeed, published studies are heterogeneous, differ in the way sexual function is assessed, and usually evaluate only one specific aspect of sexual life. Our aim was to evaluate in detail the sexual function of patients with end-stage liver disease in the waiting list for liver transplantation (LT) and to compare it to that of a controlled group from the general population matched by age and gender.
Methods
Changes in sexual functioning questionnaire, Short Form 36 Health Survey and the Hospital Anxiety and Depression Scale were used to evaluate SD, QOL and psychiatric comorbidity, respectively. Clinical data as well as a complete set of sexual hormonal profile were obtained. Controls were given the same questionnaires.
Results
Fifty three patients, 68% men with a median MELD 18, were included and compared to 22 controls. 96% had SD, which was more severe in older patients, those using spironolactone, and those suffering from anxiety. QOL was significantly impaired compared to controls. Central hypogonadism and hyperestrogenemia was present in most men. Blood levels of sexual hormones were similar in the alcoholic liver disease group compared to those of other etiology. In addition, low levels of DHEA-S were found in 97% of men. Total cholesterol and fractional cholesterol, precursors of sexual hormones correlated significantly with the level of total and free testosterone, free androgen index, SHBG and DHEA-Sulphate.
Conclusion
SD, an infra-estimated condition, is extremely common in cirrhotic patients awaiting LT. SD is likely a key factor in the impaired QOL typical of these patients. Factors associated with worsening of sexual function include advanced age, chronic spironolactone use and presence of anxiety disorders. Besides central hypogonadism, the reduced levels of DHEA, possibly due to adrenal dysfunction, is an aspect that deserves further investigation. Sexual dysfunction could, in part, be another manifestation of the recently coined 'hepatoadrenal syndrome'.

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Fifteen years of experience with intramuscular testosterone undecanoate for substitution in male hypogonadism: beneficial effects on the metabolic syndrome and high safety profile

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Background
A reliable form of androgen substitution therapy in terms of favorable kinetics and tolerance as well as effective restoration of androgenicity is paramount for hypogonadal men. The i.m. injection of the long-acting ester testosterone undecanoate (TU) offers a convenient modality for testosterone substitution.
Methods
We report data from 334 patients (147 with primary (including 38 Klinefelter patients), 100 with secondary hypogonadism and 87 with late-onset ('mixed' or 'metabolic' hypogonadism) aged 15 to 72 years (mean 42 ± 15 years) receiving altogether 6596 i.m. injections of 1000 mg of TU during a maximal treatment time of 15 years, overall corresponding to 1403 treatment years.
Components of the metabolic syndrome were assesed in 269 men receiving 4296 injections.
Results
Individual dosing intervals ranged from 10 to 14 weeks Serum T concentrations increased from 5.8 to stable 16.1 nmol within the first year of treatment. The proportion of men fulfilling the new Harmonized Criteria for definition of the metabolic syndrome decreased from initially 88 to 52% within 2 years (χ^2 for trend: $P < 0.001$). During the maximal duration of treatment, an overall favorable change from baseline was visible for a multitude of parameters related to androgen effects/metabolic risk (see Table). Prostate size increased from 16.1 ± 5.2 to 21.1 ± 5.2 ml ($P < 0.001$), whilst PSA levels moderately (1.8 ± 0.4 to 1.9 ± 0.4 μg/l, $P = 0.001$). No case of prostate cancer was observed. Hematocrit was significantly elevated during treatment but remained within the normal range (40.9 ± 2.1 to $46.2 \pm 2.5\%$, $P < 0.001$), except for occasional measurements (maximal value 56.6%). One patient suffered from deep vein thrombosis, one from stroke. No case of prostate cancer was observed.

Table 1

Parameter	Baseline	Endpoint: 15 years	*P* for ANOVA, last observation carried forward
Body mass index (kg/m^2)	31.8 ± 5.2	24.4 ± 3.2	<0.001
Waist circumference (cm)	114 ± 10.5	94.1 ± 8.7	<0.001
Weight (kg)	103.0 ± 16.3	79.1 ± 12.6	<0.001
LDL-cholesterol (mg/dl)	157 ± 29	110 ± 19	<0.001
HDL-cholesterol (mg/dl)	38.4 ± 9.7	53.6 ± 11.7	<0.001
Triglycerides (mg/dl)	198 ± 33	145 ± 21	<0.001
Fasting glucose (mg/dl)	118.1 ± 29.7	91.2 ± 15.2	<0.001
RR systolic (mmHg)	148 ± 14	128 ± 11	<0.001
RR diastolic (mmHg)	98 ± 11	81 ± 10	<0.001
Pulse (bpm)	89 ± 9	75 ± 8	<0.001

Conclusion
Intramuscular injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men of a wide age-range, substantiated by more than one decade of experience, facilitating a decrement of metabolic/cardiovascular risk factors.

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Metabolic syndrome correlates with prostate volume and biochemical and ultrasound signs of prostate inflammation in male members of infertile couples

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Introduction
The impact of metabolic syndrome (MetS) on male infertility has been poorly studied. We recently reported that MetS is associated with hypogonadism, poor sperm morphology and testis ultrasound inhomogeneity. However, the possible associations between MetS and prostate-related symptoms and signs are still lacking.
Methods
Out of 351 subjects without genetic abnormalities previously evaluated for couple infertility clinically and by scrotal ultrasound, 171 underwent also transrectal ultrasound and are here analysed. MetS was defined according to NCEP-ATPIII classification. All men underwent hormonal (including total testosterone (TT) and insulin), seminal and transrectal ultrasound evaluation. Prostate-related symptoms were assessed by National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) and International Prostate Symptom Score (IPSS).
Results
Out of 171 patients, 22 (13%) fulfilled MetS criteria. In an age-adjusted regression model, insulin levels increased as a function of MetS components ($\beta=0.134$, $P<0.0001$) and showed an inverse correlation with TT (adj.$r=-0.359$, $P<0.0001$). In an age-TT-insulin-ordinal regression model, normal morphology was negatively related to the number of MetS components ($\beta=-0.083$, $P<0.02$). At univariate analysis, the number of MetS components was weakly associated with NIH-CPSI void subdomain ($r=0.110$, $P<0.05$) and IPSS ($r=0.143$, $P<0.05$) score, however not confirmed after adjusting for confounders. Using an age-TT-insulin-adjusted model, a positive correlation between the number of MetS components and sIL-8 levels was observed ($\beta=0.760$, $P<0.05$). When transrectal ultrasound features were evaluated, using an age-TT-insulin-adjusted ordinal regression model, the number of MetS components was positively related to prostate volume ($\beta=0.086$, $P<0.0001$), arterial prostatic peak systolic velocity ($\beta=0.148$, $P=0.02$), prostate inhomogeneity severity ($\beta=0.521$, $P<0.01$) and prostate calcification size ($\beta=0.087$, $P<0.02$). No association between MetS and seminal vesicles features was found.
Conclusions
In men with couple infertility, MetS is positively associated with a higher prostate volume, biochemical (sIL8) and ultrasound signs of prostate inflammation, but not with prostate-related symptoms.

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Identification of vitamin D (VDR) and retinoic X (RXR) receptor in normal and neoplastic human reproductive tissues

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Background
Vitamin D is an important modulator of cell growth, differentiation and death in normal and neoplastic cells. Its actions are mediated by vitamin D receptor (VDR), which heterodimerizes with nuclear retinoid X receptors (RXRα). Recently, it has been suggested that vitamin D system have a role in male reproduction. The aim of this study was to investigate the VDR and RXRα localization in normal and neoplastic human male reproductive tissues.
Methods
Identification of VDR and RXRα was performed by immunohistochemistry and immunofluorescence on epididymis, seminal vesicle, testis and prostate healthy tissues and on two tissue micro arrays (TMA) platforms. TMAs were built using the most representative areas from testicular germ cell cancer (TGCTs) samples (15 carcinomas *in situ*, 25 embryonal carcinomas, 39 seminomas, 5 choriocarcinomas, 15 teratomas, and 15 Yolk sac tumours) and from 40 cases of prostate cancer (PC).
Results
VDR and RXRα were expressed in epididymis, seminal vesicle and prostate tissues. VDR showed membrane-cytoplasmic immunoreactivity while RXRα showed a cytoplasmic localization. In normal testis, VDR was expressed in Sertoli cells and in epithelium of *rete testis* while RXRα was detected in Leydig, Sertoli and spermatogonia cells. Membrane VDR immunostaining was strong in differentiated TGCTs while RXRα localization was much more positive in undifferentiated TGCTs. Notably, VDR showed high expression in healthy prostate (80%$\pm$0) compared to PC tissues (64%$\pm$16.8). Moreover, VDR expression decreased in tumours with high Gleason score (GS) (67% in GS$<$7; 50% in GS$=$7 and 30% in GS$>$7) and in these latter VDR was localized exclusively on membrane.
Conclusions
VDR and RXR are expressed in different tissue of the reproductive system and tumours, suggesting a role of vitamin D in reproduction and/or in the development of tumors. Membrane VDR localization suggest a non-genomic role of VDR in prostate and testis tumour tissues.

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Fat boosts, while androgen receptor activation counteracts, BPH-associated prostate inflammation

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Background
Metabolic syndrome (MetS) and benign prostate hyperplasia (BPH) are often comorbid. Chronic inflammation, a determinant pathogenic factor for BPH, is a putative link between the two conditions.
Methods
In a multi-center cohort of BPH patients ($n=244$) who underwent prostatectomy, we evaluated whether MetS is associated with prostatic inflammation in BPH specimens. In addition, we investigated the *in vitro* inflammatory effects of metabolic insults on human prostatic myofibroblastic cells (hBPH).
Results
Inflammatory infiltrates score (IS) in prostatectomy specimens showed a step-wise association with the number of MetS factors present ($P=0.001$). After adjusting for age, reduced HDL cholesterol and elevated triglycerides were the only factors significantly associated with IS. Increased IS was also significantly associated with hypogonadism. In an age- and testosterone (T) -adjusted model, dyslipidaemia was still associated with IS.
To investigate whether metabolic factors could directly trigger prostate inflammation, we performed preliminary studies in myofibroblastic hBPH. Among the different factors, oxidized low-density lipoprotein (oxLDL) showed the highest secretion of IL-8 ($>$10-folds) – a surrogate marker of prostate inflammation-as well as IL-6, and bFGF. Co-treatment with DHT significantly inhibited oxLDL-induced secretion of IL-8, whilst an AR-antagonist, bicalutamide, reversed DHT effects. DHT suppresses oxLDL receptor (LOX-1) expression.
Conclusions
Our data suggest that fats and insulin could have a detrimental effect on prostate health, boosting inflammation, a key pathogenic factor in BPH. Conversely, beneficial effects of DHT in counteracting lipid- and insulin-induced prostatic alterations, suggest that T – via its conversion into DHT – may have unexpected beneficial effects on prostate health.

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CATSPER calcium channels in human spermatozoa and their role in responsiveness to progesterone (P)

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CATSPER is a family of sperm-specific calcium channels activated by P in human spermatozoa (Lishko *et al.* 2011, Strunker *et al.* 2011). KO mice for CATSPER are infertile due to severe defects in sperm motility.
We studied the involvement of CATSPER in human sperm motility and P responsiveness.
Western blot analysis with an anti-CATSPER1 antibody demonstrated the presence of three major bands corresponding to CATSPER1, 2 and 3–4. By immunoflorescence we observed that channels are mainly located in the principal piece of the tail. Higher levels of CATSPER were found by flow cytometry analysis in swim up selected spermatozoa respect to unselected (50.9 ± 16.6 vs 23.4 ± 10.7, $n=6$, $P=0.01$). To investigate the role of CATSPER channels in human sperm motility, we evaluated the effects of the specific inhibitor NNC55-0396 (10 and 20 μM) and the non specific inhibitor mibefradil (30 and 40 μM) on swim up selected spermatozoa ($n=13$) by CASA system. Both compounds significantly inhibited several motility-parameters (VAP, VCL, VSL, BCF and STR). We evaluated the effect of mibefradil and NNC55-0396 on P (10 μM)-stimulated AR in swim up selected human sperm ($n=11$). Mibefradil at 30 μM was ineffective, whereas a 50% inhibition was observed at 40 μM. Conversely, NNC55-0396 compound, tested at 10 μM concentration, inhibited P-induced AR of 70%.
These results indicate that CATSPER calcium channels are involved in human sperm motility and P-induced AR. In light of a recent study (Jin *et al.* 2011) demonstrating that physiological AR occurs during transit in the cumulus matrix of the oocyte (where P is present at μM concentrations) before sperm attachment to the zona pellucida, our data suggest that CATSPER may be considered a possible molecular target for the development of novel therapeutic strategies for male infertility as well as for male-directed contraception.

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Impaired masturbation induced erections: a new cardiovascular risk factor for male subjects with sexual dysfunction

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Introduction
Erectile dysfunction (ED) is considered an early surrogate marker of silent, or even overt, cardiovascular diseases (CVD). However, epidemiological studies take into account only sexual intercourse-related erections. Although autoeroticism is a very common practice, data on masturbation-induced erections as a possible predictor of major adverse cardiovascular events (MACE) are lacking.
Aim
To evaluate the clinical correlates of impaired masturbation-induced erections and to verify the importance of this sexual aspect in predicting MACE.
Methods
A consecutive series of 4031 male patients attending the Outpatient Clinic for sexual dysfunction for the first time was retrospectively studied. Among these subjects, 64% reported autoeroticism during the last 3 months and only this subset was considered in the following analyses. In the longitudinal study, 862 subjects reporting autoeroticism were enrolled.
Main outcome measures
Several clinical, biochemical and instrumental (PGE_1 test and penile color Doppler ultrasound) parameters were studied.
Results
Subjects with an impaired erection during masturbation (46% of those reporting autoeroticism) had more often a positive personal or family history of CVD, a higher risk of reduced intercourse- and sleep-related erections, hypoactive sexual desire and perceived reduced ejaculate volume, and impaired PGE_1 test response. Prolactin levels were lower in those having impaired erection during masturbation. In the longitudinal study, unadjusted incidence of MACE was significantly associated with impaired masturbation-induced erections. When dividing the population according to the median age and diagnosis of diabetes, the association between impaired masturbation-induced erections and incidence of MACE was maintained only in the youngest (<55 year-old) and in non-diabetic subjects, even after adjusting for confounders (HR = 3.348 (1.085–10.335), $P=0.032$ and HR = 2.108 (1.002–4.433), $P=0.049$; respectively).
Conclusions
This study indicates that, in subjects with male sexual dysfunction, evaluating an often neglected sexual parameter, such as masturbation-induced erections, can provide further insights on forthcoming MACE in particular in 'low risk' subjects.

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PSA as a marker of testosterone biological activity

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Objective
To verify whether, in a large sample of male subjects seeking medical care for sexual dysfunction (SD), prostate-specific antigen (PSA) might represent a reliable marker of testosterone bioactivity, even downstream to its receptor binding.
Design
Cross-sectional study.
Methods
A consecutive series of 3156 patients attending our Unit for SD studied. Among them, only subjects without history of prostate disease and with PSA levels <4 ng/ml ($n=2967$) were analyzed. Several clinical and biochemical parameters were studied.
Results
Receiver operating characteristic curve analysis for predicting severe hypogonadism (T < 8 nmol/l) showed an accuracy of PSA = 0.612 ± 0.022 ($P<0.0001$), with the best sensitivity and specificity at PSA <0.65 ng/ml (65.2 and 55.5% respectively). After adjusting for age, low PSA was associated with higher prevalence of hypogonadism-related clinical features, (i.e. delayed puberty, lower testis volume), and associated conditions, as metabolic syndrome (HR = 1.506 (1.241–1.827); $P<0.0001$), type 2 diabetes (HR = 2.044 (1.675–2.494); $P<0.0001$) and cardiovascular diseases (HR = 1.275 (1.006–1.617); $P=0.045$). Furthermore, low PSA was more frequently associated with sexual symptoms, such as impaired sex- and sleep-related erections. The association between low PSA and hypogonadal symptoms and signs as well as with metabolic syndrome was retained even after adjusting for T levels.
Conclusions
PSA is a reliable marker of T biological activity and it may represent a new tool in detecting clinically relevant hypogonadism. The single, costless determination of PSA levels might give insights not only on the circulating levels of total T, but also on its active fractions and, most importantly, in androgen receptor bioactivity.

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Effects of testosterone replacement therapy on hepcidin levels in young hypogonadal men

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Erythrocytosis is a frequent adverse event associated with testosterone (T) administration in aged hypogonadal men, but the mechanisms involved remain poorly understood. T administration to aged men reduced hepcidin (Hpc) levels, a cytokine regulating iron availability, suggesting a potential role in deregulated erythropoiesis. Aim of present study was to evaluate the effects of T replacement therapy on Hpc levels in young hypogonadal men.

Methods
Fifty-eight subjects (18–36 years) with hypogonadism due to Kallmann syndrome (16), idiopathic hypogonadism (12), multiple pituitary deficiency (7), Klinefelter syndrome (18), and anorchidism (5) were studied. Blood samples were obtained basally (pts. never treated or after 3-months suspension) and after 6–12 months of T substitution therapy with 1 g of T undecaonate (TU) i.m. every 10–12 weeks (30 cases), or 250 mg of T enanthate (TE) im every 2–3 weeks (20 c), or 50–100 mg T gel/d (6 c). In all samples haematocrit (Ht), Hpc (by Elisa) and T (by RIA) were determined. T therapy increased T and Ht, and decreased Hpc levels ($P<000.1$). Hpc levels were negatively related to T and Ht. Fifteen subjects (26%) developed polycythaemia (Ht > 50); 10 out 15 (67%) received TE.
Conclusion
Erythrocytosis is also frequent in T treated young hypogonadic. TE administration seems favour polycythaemia respect to other formulations. T-induced suppression of Hpc may contribute mechanistically to stimulate erythropoiesis.

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P660

D3 levels and hypogonadism in men

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Objective
25-OH vitamin D3 (D3) possibly affects testosterone production. In some cross sectional studies the level of D3 correlates positively with total testosterone (TT) and free androgen index (FAI). In the European Male Ageing Study positive association of D3 with secondary and compensated hypogonadism was confirmed.
Aim
The aim of the study was to determine the relationship between D3 levels and levels of TT, LH, FSH and FAI in men. Secondary aim was to assess seasonality of measured values of D3, TT a FAI.
Methods
In 72 men with average age 68 years admitted to 5th Deparment of Internal Medicine we determined levels of D3 by chromatograhy, TT, LH and FSH by electrochemiluminescence and calculated FAI (TT/SHBG×100). Patients with severe liver disease, malignancy, antiandrogen therapy and vitamin D3 supplements were excluded. The correlation statistic was done by MedCalc system for all measured values and specifically for values in physiological range.
Results
The average level of TT was 13.3 nmol/l (interval 1–31.7 nmol/l), FAI 37% (10–88.2%), FSH 9.3 IU/l (1.3– 41.1), LH 7.3 IU/l (2.1–34.1) and D3 23.1 μg/l (4 52.5 μg/l). We did not cofirme correlation of D3 with androgen levels in whole group. In subgroup with values in physiological range we found negative correlation of D3 with FSH ($r=0.11$, $P=0.02$) and LH ($r=0.1$, $P=0.02$). We found insignificantly higher levels of TT in summer comparing with winter but we did not find any seasonality in FAI and D3 levels.
Conclusion
In our study we did not confirme correlation of D3 with TT, FAI, FSH and LH in whole group but we confirmed negative correlation of D3 and FSH and LH in subgroup with values in physiological range. In this sample, we confirmed association of D3 levels and compensated hypogonadism similarly as in European Male Ageing Study.

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P661

Changes of neuroactive steroids caused by the smoking discontinuance

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Introduction
Tobacco dependence is considered to be a serious psycho-social problem. Chronic smoking can cause fertility disorders with both sexes. The literature brings up contradictory results in relation to the effects of the smoking on the steroid hormones levels. Only a few papers discuss the effect of the nicotine addiction treatment.
Methods
We observed 26 smokers who decided to quit smoking and abstained from smoking for one year. With everyone thorough anamneses were written down, basic anthropometric data measured and at the smoking period their blood samples taken. Further samples were taken after 6, 12, 24 and 48 weeks starting with the beginning of the smoking abstinence. We measured anthropometric data, levels of steroid hormones and their neuroactive metabolites by GC-MS, LH, FSH, SHBG ing every etabolites by GC-MS, LH, FSH, SHBG using RIA during every examination. The abstinence was verified measuring the CO amount in the exhaled air and determining cotinine in their urine. The local Ethics Committee approved the study. All patients have signed an informed consent form before taking part in the study.
Results
We observed the effect of smoking abstinence on the steroid spectre with the men successful in abstaining from smoking. After a one-year abstinence the statistically significant increase in BMI with former smokers have been observed, along with the statistically significant decrease in SHBG that decreased only after 6 weeks and stayed constant. Testosterone and some other androgens had been decreasing continually during the whole first year of abstinence. Also FSH decreased along with the decreasing testosterone level. The changes in SHBG and testosterone did not correlate with BMI; apparently this is a direct effect of the smoking discontinuance.
Conclusion
After the smoking discontinuance SHBG, testosterone and other androgens decreased. This indicates the complex effects of smoking.
Acknowledgement
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P662

Testosterone undecanoate 1000 mg at 3 months does not increase prostatic specific antigen level: study on over 100 patients, December 2012

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Aim
To find if testosterone undecanoat 1000 mg injection (Nebido; Bayer-Schering) has a negative effect on prostate.
Materials and methods
i) PSA (ng/ml) was registred retrospective (from files) and prospective analysis (onset 2010). ii) From over 175 patients to whom testosterone undecanoate 1000 mg, i.m. was administrated at 3 months, in the last 4 years, and to whom the prostatic volume was quantified (*Journal of Sexual Medicine***8** suppl. 7, 2011: PO-05-013), 106 PSA analysis were done before treatment. PSA was recorded (if possible) at T1–T7 (2 weeks–4 years) (see Peretianu, this Congress); at least two analysis were registred. iii) None patient with prostatic cancer was included. iv) Statistical analysis: Student's *t*-test, simple correlation, multiple regression.
Results
i) Patients: 106 men, 18–96 years, average: 61.95; median: 62. ii) Prostatic volume (cm^3): average: 37.89. iii) Average PSA: i) before treatment: 1.76; ii) post 1 year: 1.9; iii) post 2 years: 1.68; iv) post 3 years: 1.94; v) post 4 years: 2.18. iv) Statistical difference of average: nonsignificant for all the times from 2 weeks to 4 years. v) Correlation between age and PSA is: i) significant before (r1) and after treatment after 2 years but nonsignificant for 3 and 4 years (PSA did not change as age after treatment). vi) Correlation between PSA and prostatic volume was *significant*, both before and after treatment.
vii) Multiple regression test (table) shows that PSA level post testosterone does not depend on testosterone but on age, prostatic volume (before and after treatment) and PSA initial level (before testosterone administration).

Table 1 PSA levels in patient treated with testosterone undecanoate 1000 mg at 3 months till 4 years.

	T0	T1–2 weeks	T2–3 months	T3–6 months	T3 bis 9 months	T4–1 year	T5–2 years	T6 3 years	T7–4 years
Average	1.76	2.87	2.45	2.81	2.20	1.90	1.68	1.94	2.18
S.D.	1.68	3.06	2.54	3.38	3.11	1.78	1.36	1.70	2.20
No	106	14	9	12	10	85	41	30	9
T value vs T0		0.20	0.44	0.31	0.67	0.59	0.76	0.61	0.59

Table 2 Correlation age and prostatic volume with PSA in TUD treatment till 4 years.

	Correlation age, PSA					Correlation prostatic volume, PSA				
	Before treatment	At 1 year	At 2 years	At 3 years	At 4 years	Before treatment	At 1 year	At 2 years	At 3 years	At 4 years
r	0.34	0.33	0.31	0.15	0.07	0.60	0.49	0.55	0.42	1.00
P	<0.001	<0.01	0.05	>0.1	>0.1	<0.001	<0.001	<0.001	<0.05	<0.001

Table 3 Multiple regression test.

	Observations	R^2	*F*	*P*
1 year TUD	70	0.52	24.03	<0.001
After 2 years TUD	38	0.75	34.28	<0.001
After 3 years TUD	20	0.89	47.84	<0.001
After 4 years TUD	8	0.83	6.5	0.0509

Conclusions

i) Testosterone undecanoat 1000 mg injectable i.m. at 3 months does not increase significantly PSA level after 4 years administrations. ii) PSA level post testosterone is in fact dependent on age, prostatic volume before treatment and the level before treatment.

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P663

Analysing by decade, testosterone undecanoat depot injectable does not increases prostate volume. Study during up to 6 years on hypogonadic patients

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Aim

Re-analyzing the effect of injectable testosterone undecanoat depot (TUD) in hypogonadic patients.

Material-method

i) Patients: at onset 175 men with hypogonadism (median age: 64 years). ii) Distribution: by decade. iii) TUD (Nebido-Bayer-Schering) 1000 mg was injected one per 3 months i.m. iv) Prostate volume (PV) appreciated by per-abdominal ultrasound: 3.5 MHz probe, elliptical volume (cm^3), Aloka 550. v) Time of analysis: before starting testosterone (T0), after ½ month (T1), 3 (T2), 6 months (T3), 1 (T4), 2 (T5), 3 (T6), 4 (T7), 5 (T8), 6 years (T9). vi) Maximum increment from T0 was noted Δ M %. Average increment was noted ΔA %, and vii) Statistical analysis: Student's *t*-test.

Results

i) All average prostatic volume for decade in Table 1.

ii) PV at T0 increases with age, from 17.33 (19–29 years) to 47.41 (80–89 years), $P=0.0007$. iii) Considering all observations (some at 5/6 years), TUD did not increase PV significantly (average of increment = 5.51). iv) Inside a specific decade no significant increased in PV was registered: all $P>0.05$ (80–89 decades, $P=0.057$). v) In some patients, especially from 50–79 years, TUD could decrease slightly prostatic volume.

Conclusions

Considering the risk for prostate (in elderly), testosterone undecanoat 1000 mg depot injectable is a safe treatment, even after 3–6 years of administration. Precautions should be accorded to men over 80 years old, after the third administration. Further observation needed.

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P664

Male pseudohermaphroditism Leydig cell hypoplasia

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Introduction

LH receptor plays an important role in sexual development and reproductive function. Mutations of this receptor lead to the development of three clinical conditions: Leydig cell hypoplasia, hypergonadotropic hypogonadism with primary amenorrhea and familial male limited precocious puberty. The first two entities result from inactivating mutations. In Leydig cell hypoplasia, with autosomal recessive inheritance, the phenotypic spectrum correlates with the degree of residual activity of mutated receptor.

Case

Thirty six years old patient, referred for evaluation of primary amenorrhea. It presented history of pubarche at 13 years old and absent thelarche (breast development only after esto-progestative therapy), family history of primary amenorrhea in a 1st degree, paternal line, cousin. The examination showed breast development on Tanner's stage III, sparse pubic hair, female external genitalia and single, bilateral inguinal masses. Chromosome study revealed to be a 46XY individual. Analytically the patient presented FSH 15.59 mIU/ml, LH 35.71 mIU/ml, prolactin 3.1 ng/ml, estradiol 24.0 pg/ml, total testosterone 0.10 ng/ml and 17-hydroxy-progesterone 0.96 ng/ml. The MRI showed an image suggestive of vagina and in the inguinal regions images suggestive of testicles, ovaries were not identified. Human chorionic gonadotrophin stimulation test was performed with the following results: Testosterone 0.08 (0.08 ng/ml and 17-OH-progesterone 0.94 (0.41 ng/ml. The patient underwent orchiectomy. Histological exam revealed presence of testicular parenchyma devoid of germ cells and absence of Leydig cells. Search for mutations of the LH receptor is ongoing.

Discussion

The more severe forms of Leydig cell hypoplasia are characterized by predominance of female external genitalia and absence of secondary sexual differentiation in puberty. Milder forms have predominantly male external genitalia, micropenis/hypospadias, or infertility without sexual ambiguity. It is biochemically characterized by low testosterone levels, without elevated precursors, even after stimulation with human chorionic gonadotropin, and LH increased levels.

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P665

Immunohistochemical detection of Prok1 and ProkR1 in normal testis and germ cell tumour tissue microarrays

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Prokineticins (Prok1 and Prok2) are chemokine-like peptides, which regulate through two G-protein-coupled receptors, ProkR1 and ProkR2, different physiological and pathological functions. Prok1 plays a significant role in tumour development and progression in several tissues. There are few data on the involvement of Prok1 and ProkR1 in testicular cancer. Aim of this study was to evaluate the expression of Prok1 and ProkR1 in a series of testicular germ cell tumours (GCT).

Methods

We evaluated by immunohistochemistry the expression of Prok1 and ProkR1 in 2 Tissue Micro Array (TMA), containing a series of 140 GCT (90 seminoma, S, and 50 mixed non-seminomas, NS) and 30 normal tissues. We selected all representative areas of each component of NS (embryonic carcinoma, EC, yolk sac tumour, YS, chorioncarcinoma, CH, teratoma, T, and carcinoma *in situ*, CIS).

Results and conclusions

Prok1 absent in normal germ cells resulted frequently expressed in GCT (78% S, 58% NS, 83% CIS), with higher level in S than in other types ($P<0.005$). ProkR1 was poorly expressed in GCT, except for EC (50% were positive). No correlation

was observed between ligand and its receptor presence. The expression of Prok1-ProkR1 system in GCT suggests a potential role as autocrine pathway in the development and progression of a subset of these tumours.

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P666

Endocrine disruptors in seminal fluid: bisphenol A, triclosan and benzophenone-3

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Introduction
There is concern that unintended environmental exposures to numerous chemicals, including bisphenol A (BPA), triclosan (TCS) and benzophenone-3 (BP-3), all of which can be detected in serum and urine, may have adverse endocrine effects on human reproduction. Less is known about the concentrations of these chemicals in other body fluids. Here we present data, which show that these three chemicals can also be present in seminal fluid of young men.
Materials and methods
From an ongoing study on male reproductive function in young men from a general population, we selected 28 men with high urinary excretion of BP-3, which were examined in 2007 and from which both serum and seminal fluid were available for chemical analysis. Serum and semen samples were collected within one hour. Samples were analyzed for TCS, BPA, and BP-3 by TurboFlow LC–MS/MS.
Results
BPA, TCS, and BP-3 were detected in respectively 54, 18, and 29% of the seminal fluid samples in concentrations ranging from $<$LOD, 8.470 ng/ml (BPA); $<$LOD, 6.654 ng/ml (TCS); and $<$LOD, 18.236 ng/ml (BP3) respectively. Thus, some subjects had extremely high levels in seminal fluid. The five men with the highest concentrations of TCS and BP-3 in serum also had the highest concentrations of TCS and BP-3 in seminal fluid. In some men the concentrations of BP-3 were higher in the seminal fluid sample than in the serum sample.
Conclusion
BPA, TCS, and BP-3 were detected in seminal fluid from a significant proportion of young men from a general population. We do not know whether the seminal plasma content of BP-3, TCS, and BPA originate from rete testis fluid, the epididymis, the prostate or the seminal vesicles. It remains to be seen whether the presence of these chemicals in seminal fluid is associated with effects on testicular hormones, sperm cell function, or the female reproductive tract.

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P667

Sperm DNA fragmentation as assessed by TUNEL/PI: mean values in fertile men and intra individual variability

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Tests detecting sperm DNA fragmentation (SDF) could be included in the clinical management of male infertility. TUNEL/PI is an innovative version of the TUNEL assay, and it is able to distinguish between two different populations: PI brighter and PI dimmer sperm. The aims of the study were to determine the average value of SDF in recruited men of proven fertility and to verify whether SDF discriminates between fertile and subfertile men independently from semen quality. In addition, we investigated the intra-individual coefficients of variation (CV) of SDF, by comparing two consecutive measures of SDF in the same patients. We found that in 67 recruited fertile men the average value of SDF was $35.8 \pm 14.8\%$. After matching fertile ($n=58$) to 99 sub fertile men (ratio 1:(1–3)) for age and semen quality, we observed that total SDF was significantly higher in the group of patients ($44.5 \pm 917.2\%$) than in fertile subjects (36.3 ± 14.8, $P<0.005$) and such a difference was totally due to SDF of PI brighter sperm (respectively: 27.4 ± 13.0 vs 20.8 ± 9.2, $P<0.001$) since SDF in PI dimmer population was similar in the two groups. Regarding the intra-individual CVs of SDF, they were quite low ($8.9 \pm 5.8\%$, $n=11$) within 3 months and resulted lower than any conventional semen parameter. When the length between the two determinations of SDF was >3 months, the intra-individual CV for SDF increased to $17.1 \pm 15.0\%$, $n=42$. In conclusion, we demonstrated that SDF in PI brighter provides information on male infertility additional to those obtained by routine semen analysis. These results encourage us to continue the collection of fertile men to the aim of building the reference value for SDF as assessed by TUNEL/PI. In addition, we reported that within 3 months, SDF is the most stable intra-individual semen parameter.

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P668

The identification of pre-diabetes condition with ARIC algorithm, predicts long-term CV events in patients with erectile dysfunction

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Introduction
The Atherosclerosis Risk in Communities (ARIC) algorithm is one of the most efficient instruments for the prediction of incident type 2 diabetes (T2DM). Recently it has been shown to predict another relevant cardiovascular (CV) risk factor, such as chronic kidney disease.
The aim of the present study is to verify whether, in patients with erectile dysfunction (ED), the use of ARIC diabetes risk score might improve the efficacy in predicting major CV events of other CV risk algorithms specifically developed for the assessment of CV risk.
Methods
A consecutive series of 2437 men (mean age 52.5 ± 12.9 years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample ($n=1687$) was enrolled in a longitudinal study (mean follow up of 4.3 ± 2.6 years). The assessment of metabolic risk was evaluated with the ARIC algorithm. The assessment of CV risk was evaluated using the Progetto Cuore risk engine.
Results
In the cross sectional study ARIC score was inversely related with testosterone levels, sexual functioning and penile blood flow. When longitudinal sample was analyzed, higher baseline ARIC score significantly predicted MACE even when subjects with diabetes mellitus at baseline were excluded from the analysis (HR = 1.522 (1.086–2.135), $P=0.015$ for trend). In addition, among subjects classified as 'low-risk' (CV risk $<20\%$ at 10 years corresponding to $<9\%$ at 4.3 years) by Progetto Cuore, a ROC curve analysis for ARIC (vs MACE) allowed the identification of a threshold of 0.22, which had a positive predictive value for 4.3-year MACE of 9%. Applying the ARIC score (with a threshold of 0.22) to Progetto Cuore 'low risk' subjects, we could classify as 'at high risk' 89.8% of subjects with incident MACE vs 79.6% with Progetto Cuore only.
Conclusions
In patients with ED identifying pre-diabetes, even with algorithms, predicts long-term CV events.

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P669

Is pericentromeric inversion of the heterochromatic region of chromosome 9 involved in couple infertility?

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Pericentromeric inversion of the heterochromatic region of chromosome 9 has been reported in almost 4% of the cases of male infertility.
Objective
Determining the karyotype from an infertile couple scheduled to perform IVF.
Materials and methods
Slides with metaphasic chromosomes were GTG-banded according to standard protocol for cytogenetic analysis (karyotype). FISH analysis – inverted DAPI (4′-6-diamidino-2-phenylindole) staining was used to confirm the anomaly in chromosome structure.
Results
The karyotype revealed modification of the heterochromatic region of both chromosome 9 in the male partner. The karyotype for female partner was normal. In case of male partner we suspected the pericentromeric inversion of the heterochromatic region of the long arm (q) of chromosome 9. Using Inverted-DAPI staining we confirmed the inversion on both chromosomes.
Conclusion
Karyotyping followed by FISH is useful to select appropriate couples to improve the success rate of IVF.
Further investigation of the hormonal profile and seminal liquid analyses for male partner will show the involvement of the chromosomal abnormality in the hypothalamic–pituitary–testicular axis.

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P670

The hypothalamus–hypophysis–gonads axis in men in reproductive age with obesity and different pituitary adenomas

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The aim
To study of functional status of hypothalamic–hypophysis–gonads axis men in reproductive age with obesity and different pituitary adenomas (PA).
Materials and methods
We examined 44 males with obesity 1–3 degrees with associated pathology (11 of them – pro and 33 – retrospectively). Mean age of patients was 22.9 years old. Control group constituted by 20 healthy men with different age.
All patients underwent clinical and biochemical evaluations including endocrine check, lipids profile, hormonal profile (LH, FSH, prolactin, sex steroid binding globulin, and free testosterone), genitalia ultrasonography, height, body mass, waist circumference (WC), hip circumference (HC), waist–hip ratio, questioning, and other studies.
Results
On the base of etiology of adenoma we found following: non-functional PA, 34 patients (77.3%); corticotropinoma, 5 (11.4%); craniofaryngioma, 4 (9.0%); and somatotropinoma, 1 (2.3%).
Among complaints we found headache (66%), giddiness (38%), decreasing of vision (25%), irritability (20%), pain in heart (20%), heartbeats (14%), disorders of sleep (11%), growth retardation (9.5%), etc.
The frequency of neuroendocrine and metabolic disorders was: revealed endocrine encephalopathy (60%), arterial hypertension (41%), secondary hypogonadism (2%), osteopeniya (20%), dislipidemiya (20%), polydipsiya (20%), poliuriya (18%), panhypopituitarism (16%), diabetes insipidus (11%), secondary hypocorticism (9.5%), heart ishmemic disease (6.8%), osteoporosis (6.8%), erectyle disfunction (6.8%), bitemporal hemianopsiya (4.5%), nefrolitiasis (4.5%), hepergonadotrop hypogonadism (2.3%), etc.
Hormonal profile showed hypogonadotropic hypogonadism in 43 patients (98%; mean LH ranged 2.5 mIU/l, FSH 3.08 mIU/l) and significantly low total testosterone levels (mean 6.24 ng/ml), free testosterone – 12.5 ng/ml, sex-steroid-coupled globuline – 36.6 nmol/l, STH – 0.56 nmol/l, IGF1 – 64.78 nmol/l, ACTH–51.6 pg/ml, thyroxine – 121.5 nmol/l, cortisol – 504 nmol/l. Most of the patients had central obesity with BMI >35 kg/m^2. WC was in normal range 104.3 ± 7.4 cm, HC $= 85.6 \pm 5.3$ cm, whereas waist–hip ratio >1.22. Blood tests showed dyslipidemia in all patients (100%).
Conclusions
i) For patients with different pituitary adenomas and obesity more distinctive deficiency of STH, IGF1, and hypogonadotropic-hypogonadism (98%). ii) The risk of development of metabolic disorders is very high (WC >104 cm).

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P671

A case of idiopathic hypogonadotropic hypogonadism which attained remission by LH and FSH treatment

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A 17-year-old male was referred to our hospital with lack of secondary sex characteristics. His voice didn't break and he lacked of pubes and hircus. His bilateral testicular volume was small and his sense of smell was normal. There was no varicocele or pituitary adenoma. His chromosomal study showed no aberrations. Serum levels of LH, FSH, total testosterone and free testosterone were 1.05 mIU/ml, 1.68 mIU/ml, 0.26 ng/ml and <0.6 pg/ml respectively. A repeated GnRH test revealed sluggish, but significant response of LH and FSH. In contrast, human chorionic gonadotropin (hCG) stimulation test showed normal response of testosterone. With these results, he was diagnosed as idiopathic hypogonadotropic hypogonadism (IHH).
Although there is no consensus in the first-line therapy for IHH, there are some case reports indicating that IHH patients attained remission after r-hFSH and hCG replacement. In our case, response of LH to GnRH might suggest effectiveness of this therapy.
However, because he disliked frequent injections, he chose testosterone replacement therapy instead of r-hFSH and hCG replacement. After 2 months, his body hair gradually grew and his voice changed. After 13 months, in anticipation of recovery of his own hormonal secretory capacity, r-hFSH and hCG replacement therapy was introduced instead of testosterone therapy. At 6 months

after initiation of the treatment, his serum level of total testosterone reached 7.33 ng/ml, then, he discontinued replacement therapy. Finally, his serum levels of LH, FSH and testosterone had been kept within normal range and he holed sexual desire and activity.
We experienced a case in whom r-hFSH and hCG replacement therapy successfully normalized pulsatile gonadotropin secretion, serum testosterone level and sexual function. Because IHH has several variations, it is important to carefully choose a therapy depending on each case.

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P672

Risk factors associated with primary and secondary reduced libido in male patients with sexual dysfunction

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Introduction
Hypoactive sexual desire is defined as a persistent or recurrent deficient or absent sexual fantasies or desire for sexual activity which should not be comorbid with other medical conditions or with the use of psychoactive medications. Reduced libido is a symptom referring more to a reduction in sexual drive for sexual activity. The aim of the present study is to investigate the risk factors of primary reduced libido (i.e. not associated with conditions causing loss of libido such as hypogonadism, hyperprolactinemia, psychopathology and/or psychoactive medications) or secondary reduced libido (i.e. with aforementioned conditions) in male patients with sexual dysfunction.
Methods
A consecutive series of 3714 men (mean age 53.2 ± 12.5 years) was retrospectively studied. Patient's reduced libido was evaluated using question #14 of SIEDY ('Did you have more or less desire to make love in the last three months?')
Results
Reduced libido was comorbid with erectile dysfunction, premature ejaculation and delayed ejaculation in 38, 28.2 and 50% respectively, whereas it was isolated in 5.1%. Reduced libido prevalence was substantially increased by hypogonadism, almost doubled by psychopathology and universally present in subjects with hyperprolactinemia (secondary reduced libido). Subjects with primary reduced libido are characterized by higher post-school qualification, more disturbances in domestic and dyadic relationships, and an overall healthy body (lower glycaemia and triglyceride levels). Accordingly, in patients with primary reduced libido the risk of major cardiovascular events as calculated with the Progetto Cuore algorithm was lower than in the rest of the sample. Features of hypogonadism- or psychopathology-associated reduced libido essentially reflect their underlying conditions. Comorbidity with other sexual dysfunctions did not affect the main characteristics of primary or secondary reduced libido.
Conclusions
Primary and secondary reduced libido have different risk factors and clinical characteristics. Recognizing primary or secondary reduced libido will help clinicians to identify comorbidities and to tailor appropriate treatments.

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P673

The role of testosterone augmentation in patients with acute exacerbation of chronic obstructive pulmonary disease (COPD) in improving functional recovery and hospital stay

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Introduction
Low testosterone levels are found in up to 50% of male patients with chronic obstructive pulmonary disease (COPD), and acute illness is associated with further suppression of the central and peripheral gonadal axis. Testosterone supplementation in chronic stable COPD patients has yielded mixed results, demonstrating increased lean body mass, and improved exercise capacity in some studies, but not others. Use of anabolic steroids during acute illness has shown benefits in selected populations, particularly major burns patients. We studied the use of testosterone augmentation in patients with acute exacerbation of COPD, in improving functional exercise capacity and recovery during their hospitalisation.
Methods
We conducted a randomised, placebo-controlled trial in 60 consecutive patients admitted with the primary diagnosis of acute exacerbation of COPD. Patients with a secondary diagnosis of other concomitant acute illness such as myocardial infarction, or acute renal failure were excluded. At baseline, blood tests (FSH, LH, Testosterone, SHBG, Albumin, Full blood count), anthropometric measurements, BORG dyspnea scale and 6 min walk tests (6MWT) were conducted. Patients were then randomised to receive either testosterone cypionate (400 mg i.m.) or placebo on 1st day of admission. BORG dyspnea scale and 6MWT were repeated during the hospitalisation and 2 weeks after discharge. Outcome measures included duration of hospitalisation, BORG dyspnea scale and 6MWT results. Patients will be stratified by their baseline testosterone levels.
Results
Recruitment of subjects is currently still ongoing. Currently, 10 patients have been recruited. Preliminary data analysis of the subjects (age range 62–78 years) show a trend towards a shorter hospitalisation stay, and improved dyspnea scale and 6MWT results.
Conclusion
Testosterone supplementation may offer a novel therapy to improve recovery from acute illness, particularly in patients with COPD who often have a low baseline testosterone level.

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P674

Fourty six, XX male sex reversal syndrome with infertility: a case report

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Sex reversal syndrome (SRS) is a kind of human genetic disease about gender dysplasia, which is characterised by inconsistency between gonadal sexuality and chromosome sexuality. The clinical types include 46, XY female SRS and 46, XX male SRS. The early studies of human gonadal differentiation found that human Y chromosome contains testis-determining factor (TDF) found that the sex-determining region Y (SRY) gene, which is located in Y chromosome, was the best candidate gene for TDF, and confirmed that protein encoded by it played an important role in the process of sex determination. 46, XX male SRS is characterized by the presence of a 46, XX *karyotype*; male external genitalia ranging from normal to ambiguous; two testicles; azoospermia; and absence of Müllerian structures. Approximately 80% of individuals with 46, XX testicular DSD present after puberty with normal pubic hair and normal penile size, but small testes, gynecomastia, and infertility resulting from azoospermia. It is generally a result of unequal crossing over between X and Y chromosomes. Approximately 20% of individuals with 46, XX male SRS present at birth with ambiguous genitalia. Gender role and gender identity are reported as male. If untreated, males with 46, XX male SRS experience the consequences of testosterone deficiency.
We report a case of XX male with chief complaints of infertility.
A 29-year-old infertile male was referred to the Urology Department. His height was 166 cm and weight 74 kg. He had normal external male genital phenotype and secondary sex characters. No gynecomastia was noted. At physical examination soft and atrophic testes were palpated. Laboratory analysis and testis biopsies indicated nonobstructive azospermia. Serum concentrations of LH and FSH were elevated at 12.8 mIU/ml (normal range 1.2–8.75 mIU/ml) and 31.88 mIU/ml (normal range 1.3–13.8 mIU/ml) respectively. Testosterone hormones level was normal at 4.8 ng/ml (normal adult male range 2.8–11 ng/ml), as was the serum prolactin concentration at 9 ng/ml (normal range 1.2–29 ng/ml). Peripheral blood cultures from this phenotypically normal male showed a normal female. Chromosomal analysis showed 46, XX karyotype.
In conclusion, a multidisciplinary approach should be adopted in the management of 46, XX individuals. All patients with azoospermia must be karyotyped. Sperm donation remains the only fertility treatment available. The 46, XX patients need lifelong followup led by an endocrinologist with regular imaging of the gonads, bone density measurements, baseline blood tests, and testosterone supplementation. Psychological support is a key part of a holistic approach.

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P675

Investigation on psychological symptoms improves ANDROTEST accuracy in predicting hypogonadism in subjects with sexual dysfunction

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The role of psychological symptoms in recognizing late-onset hypogonadism (LOH) is still controversial. The aim of the study is to evaluate the association between LOH and specific psychological symptoms and to verify whether investigating intra-psychic domain improves the accuracy of a validated case-history tool (ANDROTEST) in detecting LOH. A consecutive series of 1009 subjects (mean age 49.23 ± 13.34) consulting for sexual dysfunction was studied. Intra-psychic symptoms were investigated by Middlesex Hospital Questionnaire (MHQ), a self-reported questionnaire for screening of mental disorders.
A minimum set of 2 MHQ items was identified through iterative ROC curve analysis, with assessment of sensitivity and specificity for hypogonadism (calculated free testosterone <0.225 nmol/l) in an exploratory sample of 462 patients. Sensitivity and specificity were verified in a validation sample of 547 subjects, in which the final 2-item version showed an accuracy of 58.4 ± 3.2% in detecting hypogonadism. The combination of the 2-item score with ANDROTEST increased the accuracy in predicting hypogonadism (0.741 ± 0.029; $P<0.0001$), when compared to ANDROTEST (0.696 ± 0.018; $P<0.0001$) and the 2-item score ($P<0.05$) alone. Hence, combining these two psychological symptoms with a physical scoring system improves its ability in detecting hypogonadism. The combination of the scores should be tested in other studies.

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P676

Two unconventional risk factors for major adverse cardiovascular events in subjects with sexual dysfunction: low education and reported partner's hypoactive sexual desire in comparison with conventional risk factors

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Introduction
The classification of subjects as low or high cardiovascular (CV) risk is usually performed by risk engines, based upon multivariate prediction algorithms. However, their accuracy in predicting major adverse CV events (MACE) is lower in high-risk populations, since they take into account only conventional risk factors.
Aim
To evaluate the accuracy of Progetto Cuore risk engine in predicting MACE in subjects with erectile dysfunction (ED), and to test the role of unconventional CV risk factors, specifically identified for ED.
Methods
A consecutive series of 1.233 men (mean age 53.33 ± 9.08 years) attending our outpatient clinic for sexual dysfunction was longitudinally studied for a mean period of 4.4 ± 2.6 years.
Main outcome measure
Several clinical, biochemical, and instrumental parameters were evaluated. Subjects were classified as high- or low-risk, according to previously reported ED-specific risk factors.
Results
In the overall population, Progetto Cuore-predicted population survival was not significantly different from the observed one ($P=0.545$). Accordingly, Receiver Operating Characteristic (ROC) analysis shows that Progetto Cuore has an accuracy of 0.697 ± 0.037 ($P<0.001$) in predicting MACE. Considering subjects at high-risk according to ED-specific risk factors, the observed incidence of MACE was significantly higher than the expected for both low-educated and patients reporting partner's hypoactive sexual desire (HSD, both <0.05), but not for other described factors. The area under ROC curves of Progetto Cuore for MACE in subjects with low-education and reported partner's HSD was 0.659 ± 0.053 ($P=0.008$) and 0.550 ± 0.076 ($P=0.570$) respectively.
Conclusion
Overall, Progetto Cuore is a proper instrument for evaluating CV risk in ED subjects. However, in ED, other factors as low-education and partner's HSD concur to risk profile. At variance with low-education, Progetto Cuore is not accurate enough to predict MACE in subjects with partner's HSD, suggesting that the latter effect is not mediated by conventional risk factors included in the algorithm.

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P677

'It takes two to tango': the relational domain in a cohort of subjects with erectile dysfunction (ED)

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Introduction
The relational domain of erectile dysfunction (ED) is difficult to investigate in a clinical setting. We developed and validated SIEDY, a 13-item structured interview, which assesses, beside the organic (Scale1) and intra-psychic (Scale 3) domains, also the relation one (Scale 2). We previously established a pathological threshold for SIEDY Scale 1 and 3.
Aim
To identify a pathological threshold of SIEDY Scale 2.
Method
A non-selected, consecutive series of 2992 subject with ED was retrospectively evaluated. In a first consecutive series of 844 patients (Sample A, studied without systematically applying a psychometric test: Middlesex Hospital Questionnaire, MHQ) a pathological threshold of SIEDY Scale 2 score was identified through receiver operating characteristic, using, as surrogate marker of impaired couple relationship, at least a positive answer to two standard questions on conflict within the couple and on the presence of extramarital affairs.
Main outcome measure
Sensitivity and specificity, along with possible associations with biological and psychological correlates were verified in a further sample of 2148 patients (Sample B).
Results
In sample A, a threshold of Scale 2 score ≥ 2 predicts couple impairment with a sensitivity of 53% and specificity of 66%, and an overall accuracy of 62.0 ± 2.2% ($P<0.0001$). When this threshold was verified in sample B, Scale 2 score ≥ 2 was associated with a higher risk of anxiety and depressive symptoms, higher prevalence of psychopathology, and higher Scale 3 scoring, even after adjusting for confounders. In the same sample, a Scale 2 score ≥ 2 was associated with a reduced intimacy during sexual intercourse and overall worse sexual functioning.

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Neuroendocrinology

P678

Genotype and phenotype characterization of the cohort of Italian patients with idiopathic central hypogonadism (ICH)

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ICH is a rare disease characterized by a complex pathogenesis, but with a strong genetic component. ICH may be associated to several other morphogenetic or inborn defects, such as the osmic defects that identify the Kallmann syndrome (KS). The description of several pedigrees including relatives affected either with isolated osmic defects or KS or normoosmic ICH (nICH) justifies the emerging idea of ICH as a complex genetic disease characterized by variable expressivity and penetrance. In order to improve the management of ICH patients we decided to create an Italian network aiming to collect and characterize a large national

patients series. Among the 363 total index cases, 151 had KS and 212 nICH. The number of familial cases in the two categories was similar (21.8% KS and 19.3% nICH), but the prevalence of associated malformations or cryptorchidism was higher in KS (20.5 or 29.8% vs 5.6 or 12.7% in nICH respectively). Genetic analysis allowed the identification of contributing genetic defects in about 32% of the patients, with a major involvement of KAL-1 (8.2%), FGFR1 (11.5%) and PROKR2 (8.7%) and a rare involvement of other candidate genes. Variants were detected on a single allele in 88% of the cases, whereas biallelic variations affecting either GnRHR or PROKR2 genes were found in 2.4% and digenic defects in the remaining 9.6%. These digenic defects were quite heterogeneous involving elements on different pathways and were distributed among KS and nICH patients. In conclusion, although accumulating evidences indicate the existence of common pathogenic mechanisms for KS and nICH, the combination of hypogonadism with associated malformations or cryptorchidism appears more frequent in KS. Systematic analyses of candidate genes indicate that the pathogenesis of both phenotypes is still largely unknown, but a multiple gene involvement may be seen in both cases.

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P679

DHEA enhances working memory and prevents distraction: behavioural and ERP evidence from an auditory–visual distraction paradigm

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Introduction
Several studies suggest DHEA and DHEAS (DHEA(S)) are related to memory enhancement and a better performance under stress. An anti-cortisol action may contribute to those relations. We looked for a new level of evidence, by studying DHEA(S) and cortisol relations to working memory (WM) and distraction in humans also at the electrophysiological level.
Subjects and methods
Twenty-eight healthy female volunteers (18–26 years old) were presented a well-established auditory–visual distraction task protocol. EEG was recorded during the performance of one task with WM load (WM1) and other without, while ignoring task-irrelevant sounds (80% standard – st; 20% novel – nov). ERPs were averaged for each auditory-stimulus trial type and WM condition. Novelty-P3 was identified in the nov minus st difference waveforms. Salivary DHEA, DHEAS and cortisol were measured before each task and at 30 and 60 min.
Results
With simultaneous WM load and distraction: i) Hit rate decrease was directly related to basal cortisol ($P<0.05$) and inversely related to DHEA reactivity (30/0 min) increase between conditions ($P<0.05$); and ii) Reaction time increase was inversely related to basal cortisol ($P<0.05$) and directly related to DHEA reactivity increase between conditions ($P<0.005$).
Regarding auditory ERPs, novelty-P3 amplitude in WM1 was directly related to cortisol/DHEA ratio before that task ($P=0.007$). In visual ERPs, P300 amplitude in WM1 was directly related to basal DHEAS ($P=0.011$) and changed due to WM load in direct relation to DHEA reactivity ($P=0.005$).
Discussion
In more demanding situations, higher basal cortisol was related to faster answers and more errors whereas DHEA reactivity presented opposite relations. At the electrophysiological level, distraction during WM load was in direct relation to cortisol/DHEA ratio and processing of the task-relevant visual stimulus was enhanced by higher basal DHEAS and DHEA reactivity. Overall, higher cortisol level was related to worse performance and more distraction while DHEA(S) showed opposite effects.

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P680

High glucose induces hypogonadotropic hypogonadism by interfering with GPR54 signaling in the preoptic area of the hypothalamus

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The metabolic syndrome (MetS) is a clustering of metabolic and cardiovascular risk factors, having in insulin resistance the key element. In males, MetS is also associated to hypogonadism. We recently found that high fat diet (HFD)-induced MetS rabbits also developed hypogonadotropic hypogonadism, showing a reduced immunopositivity for gonadotropin relasing hormone (GnRH) in the hypothalamus. In this study, we investigated the pathogenetic link between Mets components and the onset of hypogonadism in the MetS animal model. Interestingly, low gonadotropin level significantly correlated with MetS components and elevated glucose and cholesterol levels resulted the major determinants for this association. In agreement, glucose tolerance, evaluated *in vivo* by oral glucose tolerance test (AUC-OGTT), correlated negatively with gonadotropin level. We identified a positive association between the number of factors of the MetS and GLUT4, a glucose transporter, gene expression. GLUT4 was negatively associated with gonadotropin level and positively with AUC-OGTT and hypothalamic gene expression of inflammatory markers, such as cycloxigenase 2 (COX2) and interleukin-6 (IL-6). Glucose tolerance resulted significantly associated with the mRNA expression of GPR54 in the preoptic area of the hypothalamus and in agreement, the immunopositivity for GPR54 was significantly reduced in the hypothamic sections of HFD rabbits. KiSS1/GPR54 system is a central regulator of GnRH neurons. We therefore studied the effects of high glucose in human fetus-derived GnRH-secreting neuroblasts, the FNCB4 cell line. High glucose exposure (22 and 40 mM) significantly reduced the expression of GnRH, KiSS1 and GPR54. A subset of HFD rabbits was treated with INT-747, able to ameliorate the metabolic profile. This treatment was able to increase GnRH, and to reduce, COX2 and IL-6- gene expression; without preventing HFD-related hypogonadotropic hypogonadism. In conclusion, our results suggest that negative effects of hyperglycemia on hypothalamic function may contribute to testosterone deficiency in MetS.

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P681

Hypothalamic melanin-concentrating hormone influences liver and adipose lipid metabolism

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Introduction
Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide which is located in the lateral hypothalamus and regulate the energy balance. MCH deficient mice are hypophagic, lean and do not develop hepatosteatosis when fed on high fat diet. The MCH increases food intake and adiposity, so we sought to investigate the role of the MCH on adipocyte and hepatic metabolism.
Methods
MCH were chronically administered into the lateral ventricles of rats brain, using osmotic pumps that released the MCH for a week. To study whether the sympathetic nervous system mediates the actions of MCH on white adipose tissue, deficient mice for the three beta-adrenergic receptors were used (triple knockout mice). To determine whether the central effect of MCH on the liver was mediated through the parasympathetic nervous system (PSNS), the vagus nerve was dissected. Adenoviral particles overexpressing MCH receptors (MCH-R) were estereotaxically administered into arcuate and lateral hypothalmus (LHA,

ARC). Tissues were analyzed to determine the expression of genes and proteins involved in lipid metabolism of liver and fat.
Results
The activation of MCH receptors (MCH-R) promotes the liver fat storage through the parasympathetic nervous system (PSNS), whereas it increases lipid deposition in WAT via the suppression of sympathetic traffic. These metabolic actions are independent of parallel changes in food intake and energy expenditure. Genetic activation of MCH-R increases body weight gain and food intake, specifically in the LHA modulated hepatic lipid metabolism, whereas the specific activation of this receptor in the arcuate nucleus affected adipocyte metabolism.
Conclusions
Central MCH system increases lipid storage via modulation of adipocyte and hepatic metabolism. Activation of MCH-R in the ARC control adipocyte lipid metabolism via a SNS-dependent mechanism, while the activation of MCH-R in the LHA influences hepatic lipid metabolism through the PSNS.

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P682

Hormonal therapy satisfaction is associated with better quality of life in transsexual persons

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Introduction
Quality of life in transsexual persons after transition is among the most important outcome factors.
Methods
A specialist center cross-sectional study in 193 transsexual women and 128 transsexual men (mean age 42.5 years) after on average 7.4 years of cross-sex hormone therapy and 6.6 years since sex reassignment surgery (SRS), compared to an age-and gender matched control population (1 to 3 matching). Self-reported physical and mental health using the Dutch version of the SF-12 Health survey. Medical history and postsurgical outcome were addressed by a new-developed questionnaire.
Results
Compared to age-matched control men and women, transsexual women scored worse both on physical and mental functioning (all P values <0.001). Transsexual men reported equal degree of physical functioning compared to control women, but scored worse than control men. Mental well-being in transsexual men was poorer in comparison with control men and women (all P values <0.001). In the total sample, age was negatively correlated with physical functioning, whereas educational level was positively associated. Patients with the lowest income quartile had worse physical and mental functioning compared to the others. Participants who lived with a partner had a better mental well-being. In transsexual men, having children was positively associated with mental-well being; in transsexual women the opposite was observed. Experiences of complications of phalloplasty, erection prosthesis or vaginoplasty were not associated with quality of life scores. However, satisfaction with these procedures was positively related to mental well-being. Likewise, both in transsexual men and women satisfaction with hormonal therapy was positively associated with mental and physical functioning (both $P<0.001$).
Conclusion
Results of the current study indicate transsexual men and women after long-term cross-sex hormone treatment and SRS report worse mental well being compared to a control population. QOL in transsexual persons showed a strong association with treatment satisfaction, social and economical determinants.

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P683

The small GTPase Rab18 modulates neuroendocrine secretion by interacting with components of the microtubule-based secretory granule transport machinery

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Several Rab proteins control secretory granule trafficking and, hence, hormone release, by regulating the activity of different components of the secretory granule transport machinery. Particularly, Rab18 inhibits secretory granule movement, which leads to reduced neuropeptide and hormone secretion in PC12 pheochromocytoma cells and ACTH-secreting ATt20 cells, respectively, but how this GTPase accomplishes its role and the identity of the proteins that regulate Rab18 activity (effectors) remain unknown. In this work, we searched for the Rab18 effectors that ultimately determine its participation in neuroendocrine secretion. Time-lapse video-microscopy revealed that a functional microtubule-based cytoskeleton network is necessary for Rab18 to anchor to the secretory granules and to inhibit their movement. Furthermore, yeast two-hybrid experiments allowed us to identify a plethora of putative Rab18 interacting proteins, among which it is worth to highlight a subunit of the microtubule-associated molecular motor kinesin-1 for its well-known role in anterograde secretory granule transport. We confirmed Rab18/kinesin-1 interaction by Fluorescence Resonance Energy Trasnfer (FRET), which also showed that such an interaction only occurs with the active, GTP-bound conformation of Rab18. Furthermore, FRET also revealed that huntingtin (HTT), a protein that modulates intracellular membrane trafficking by recruiting the molecular motors dynein or kinesin to the vesicle surface, associates with the inactive, GDP-bound form of Rab18. On the other hand, HTT overexpression increased Rab18 association to the surface of secretory granules in PC12 cells, which suggests that HTT could act as a guanine exchange factor (GEF) for this Rab GTPase. Altogether, our results suggest that Rab18 reduces neuroendocrine secretion by interacting and regulating the activity of various components of the microtubule-based transport apparatus.

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P684

Roles of kisspeptin partners, NKB and dynorphin, in the control of gonadotropin secretion: revisiting the KNDy paradigm

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KNDy neurons, which co-express kisspeptins, neurokinin B (NKB) and dynorphin (Dyn), play a key role in the tonic control of gonadotropin secretion by modulating the GnRH release. The assumed KNDy model proposes that NKB and Dyn (as stimulatory and inhibitory signals, respectively) auto-regulate the secretion of kisspeptins onto GnRH neurons. However, some aspects of this paradigm remain poorly defined. In this context, the aims of this work were to characterize i) the putative effects of NKB signaling in the control of FSH secretion ii) the role of Dyn tone in shaping gonadotropin secretion and responses to NKB.
For the *first goal*, the effects of NKB agonist, senktide, on FSH release were explored across rat postnatal development; LH responses to senktide and FSH responses to kisspeptin-10 (Kp-10) were also assayed. Pre-pubertal rats displayed FSH (as well as LH) responses to central injection of senktide; FSH responses to Kp-10 were also detected at this age. In clear contrast, adult females were totally irresponsive to senktide in terms of FSH release, despite their proven LH responsiveness to NKB. In turn, adult males, which are highly responsive to Kp-10, did not display FSH (or LH) responses to senktide.
For the *second goal*, gonadotropin responses to NKB stimulation were explored in adult rats after blockade of Dyn receptors (KOR) with the antagonist, nor-BNI. Adult male rats became responsive to senktide in terms of stimulation of LH secretion after KOR blockade. Moreover, female rats displayed unambiguous FSH responses to senktide after treatment with nor-BNI.
In sum, we have documented i) the divergent patterns of gonadotropin (FSH vs LH) responses to NKB and Kp-10 stimulation ii) the role of Dyn signaling in inhibiting basal gonadotropin secretion and their responses to NKB. All in all, our data contribute to refine our present understanding on how different elements of the KNDy node participate in the dynamic and differential control of the secretion of both gonadotropins in both sexes and at different stages of postnatal maturation.

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P685

Antipsychotic-induced hyperpolactinemia: clinical particulars and relation to sexual dysfunction

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Objectives
To assess prevalence of antipsychotic-induced hyperpolactinemia (AIH) in psychiatric in-patients and to describe its clinical characteristics and association of AIH with sexual function.
Methods
A cross-sectional study in 143 consecutive psychiatric in-patients (F/M = 65/78), mostly with schizophrenia (93%), currently taking antipsychotics. The patients were screened for serum prolactin, sex hormones, gonadotropin levels and macroprolactin. For assessment of any sexual dysfunction, UKU side effects rating scale (UKU), Psychotropic-Related Sexual Dysfunction Questionnaire (PRSexDQ) were used.
Results
Overall prevalence of AIH was 57.0% (F, 72.0%; M, 43.6%). Macroprolactin was extremely rare (one patient only, 2.0%). AIH was asymptomatic in 19% of females and in 54% of males. Hyperprolactinemic women had a higher rate of menstrual dysfunction (56 vs 14%, $P = 0.006$) and galactorrhea (66 vs 0%, $P < 0.001$), compared to normoprolactinemic. Prolactin level inversely correlated with that of estradiol ($R = -0.35$, $P = 0.03$) and correlated positively with PRSexDQ score of questions on the impact of sexual dysfunction on quality of life ($R = 0.35$, $P = 0.02$). For assessment of sexual dysfunction, males were divided into two age groups: 19–31 and 32–45 years. Hyperprolactinemic men in the younger age group had a higher UKU score of questions on increased sexual desire ($P = 0.026$) compared with normoprolactinemic of the same age group. In the younger, but not in the older age group, prolactin level correlated positively with UKU score in questions about reduced sexual desire ($R = 0.35$, $P = 0.029$). There were no association between AIH and weight gain and/or obesity in patients of both genders.
Conclusions
AIH found by screening is more than 1.5-fold prevalent than that diagnosed by referral. In patients with AIH, measurement of macroprolactin is unnecessary. AIH cause menstrual disorders (oligomenorrhea and amenorrhea), galactorrhea and decreased estradiol level in females. AIH per se does not lead to weight gain and obesity. AIH is associated with sexual dysfunction in females, affecting their quality of life. AIH is associated with change of libido (increasing or reducing) in males 19–31 years old.

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P686

Low carbohydrate/high fat energy intake decreases estrogen receptor alpha expression in the arcuate nucleus of the rat hypothalamus

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Low-carbohydrate/high fat diets (LC-HFD) effectively induce body weight loss in both animals and humans; however, in rats this is paralleled by increased visceral adiposity contributing to impaired glucose tolerance. Estrogen inhibits adipose tissue accumulation, with estrogen deficient female animals displaying increased body weight and visceral adiposity with reduced insulin sensitivity, which can be reversed with estrogen replacement. Consistent with the effects seen in females, estrogen supplementation also improves insulin sensitivity in male animals. Within the hypothalamus, the arcuate nucleus (ARC) is a key area of metabolic control. To test the hypothesis that central estrogen signalling plays a role for the phenotype observed with LC-HFD in rats, we investigated whether pair-feeding isoenergetic amounts of two different LC-HFD affects estrogen receptor alpha (ERα) expression in the ARC. Male Wistar rats (12-week old) were isoenergetically pair-fed on chow (CH), 'Atkins-style' LC-HFD1, (protein matched to chow, 78.7/19.1/2.2); and ketogenic LC-HFD2 (low protein content, 92.8/5.5/1.7) (% of metabolisable energy, fat/protein/carbohydrate) for 4 weeks. Rats were perfused with 4% paraformaldehyde and excised brains were cryosectioned at 30 µm and immunohistochemistry was used to visualize ERα expression. Overall, ERα was selectively expressed in the ARC. When comparing the different diet groups, immunohistochemistry revealed a decreased expression of ERα in the ARC of both LC-HFD1 and LC-HFD2 when compared to CH rats. There was no difference between LC-HFD1 and LC-HFD2. In conclusion, due to the fundamental role of estrogen in the control of adiposity and insulin sensitivity, our findings suggest that central expression of ERα is regulated by the macronutrient composition of a diet. Furthermore, the phenotype observed with LC-HFD may be related to decreased ERα expression in the ARC.

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Digenic and oligogenic cases in a large cohort of idiopathic central hypogonadism (ICH) patients

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ICH is a rare and heterogeneous condition due to defects in the onthogenesis, migration and action of GnRH secreting neurons. Recent publications indicate that ICH, though characterized by a strong genetic component, is a disease of multifactorial origin. Indeed, digenic and oligogenic defects have been described as a possible pathogenic explanation for this disease. Among the cohort of 315 ICH patients we identified 3 KS and 7 nICH patients (7 males, 3 females) with a biallelic defect. These oligogenic defects were quite heterogeneous involving elements on different pathways. First familial case (brother and sister) present a compound heterozygosity on the same gene, the GnRHR, the second pedigree show a duplication in KAL1 and a variant in FGFR1, while the other 7 show biallelic variants on different genes: PROKR2 and GnRHR; PROKR2 and PROK2, FGFR1 and SEMA3A, GNRHR and FGF8, FGFR1 and PROKR2 in 3 cases. In this small group of patients, we observe a suggestive enrichment for complex phenotypes, such as mono or bilateral cryptorchidism, midline defects and synkinesia. One case, with PROKR2/PROK2 variants, showed a permanent reversal of the ICH after 6 years of testosterone replacement therapy. Putting together the genetic and phenotypic data we could observed a more severe phenotype in patients carrying a biallelic defect involving the FGFR1 gene. Five out of ten patients were presenting a genetic variation on PROKR2 or FGFR1 gene, thus suggesting that single heterozygous mutations in these genes might represent frequent causes of genetic susceptibility to ICH which would need another hit to become manifest. Finally, mutations on GnRHR gene appear interestingly associated with the less severe phenotypes.

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The characterization of sleep–wake pattern and its association with melatonin, as a marker of the circadian function of the suprachiasmatic nucleus, in craniopharyngioma patients

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Hypothesis
We hypothesised that damage to the hypothalamus by local tumour, surgical treatment, or irradiation might involve the suprachiasmatic nucleus and thereby melatonin secretion, leading to disturbed circadian function and clinical manifestations such as daytime sleepiness and fatigue in craniopharyngeoma patients.
Objective
We aimed to assess the influence of craniopharyngiomas and their treatment on melatonin secretion, sleep pattern, sleep quality, fatigue, and sleepiness.
Subjects and measures
We included 15 patients with craniopharynioma and 15 gender, age, and BMI matched healthy controls. Salivary melatonin and cortisol were measured over a 24 h-period. Sleep–wake patterns were characterized by two weeks of actigraphy recordings and sleep diaries. Sleepiness, fatigue, sleep quality, and general health were assessed by the four questionnaires: i) 'Multidimensional Fatigue Inventory'; ii) 'Pittsburgh Sleep Quality Index'; iii) Epworth Sleepiness Score; and iv) 'Short Form-36'.
Results
The patients had lower general health ($P = 0.01$), increased mental fatigue ($P = 0.05$), increased daytime dysfunction ($P = 0.05$), increased sleep latency ($P = 0.04$),

and tended to have increased daytime sleepiness, general fatigue, and impaired sleep quality compared to healthy controls (all $P \leq 0.08$). Patients had lower AUC-melatonin ($P = 0.04$) and higher evening cortisol concentrations. Low midnight melatonin was associated with reduced sleep time ($P = 0.03$) and efficiency ($P = 0.02$), and borderline to increased sleepiness, impaired sleep quality, and physical health ($P \leq 0.08$). High midnight cortisol levels were associated with an increased number of awakenings ($P = 0.02$) and sleep time ($P = 0.07$). Midnight melatonin remained independently related to sleep time after adjustment for cortisol.
Conclusion
Our data indicated a relationship between low midnight melatonin and reduced sleep time, impaired sleep efficiency, and reduced physical activity in craniopharyngioma patients. This might be due to the influence of craniopharyngioma on the sleep regulatory nuclei.

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Pituitary adenoma and associated tumors

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Objective
It has been difficult to identify factors that affect the risk of cancer but we know that people are at higher risk as they get older, or have a strong family history of cancer. The potential influence of environmental and behavioral factors remains poorly understood.
Aim
To study associations between PA and family history of malignancy in first-degree and second-degree relatives. As separate analysis, to study associations between PA patients diagnosed with cancer and their family history of malignancy.
Patients and methods
We reviewed clinical records for 485 patients with PA (acromegaly, $n = 121$; nonfunctioning PA-NFPA, $n = 206$; prolactinoma, $n = 158$) from the period 2000–2012.
Results
Fifty percent of patients with PA had a family member affected with cancer. Thirty-four percent of NFPA patients and 27% of acromegalics had the first-degree relative(s) with cancer, significantly higher than patients with prolactinoma (19%; $P < 0.006$). On the contrary, 41% of patients with prolactinoma had the second-degree relative(s) with cancer, significantly higher than patients with NFPA or acromegaly (23 and 25% of patients, respectively; $P < 0.0001$). According to PA type, 16% of patients with prolactinoma had a family member with breast cancer, significantly higher than patients with NFPA (11%) or acromegaly (7%, $P < 0.05$). 11% of patients with prolactinoma had a family member with colorectal cancer, significantly higher than patients with NFPA (6%) or acromegaly (4%; $P < 0.05$). Separate analysis of PA patients diagnosed with cancer and their family history of malignancy, shows that 39/485 PA patients had cancer of any type (acromegaly, 13/121; NFPA, 17/206; prolactinoma, 9/158). Eighteen of these PA patients diagnosed with cancer had a strong family history of malignancy, particularly NFPA patients (NFPA, $n = 14$; acromegaly, $n = 2$; prolactinoma, $n = 2$; $P < 0.05$).Twenty-one PA patients diagnosed with cancer did not have a family history of malignancy (NFPA, $n = 3$; acromegaly, $n = 11$; prolactinoma, $n = 7$).
Conclusions
i) The results suggest presence of associated tumors in families of patients with PA (in 50%); ii) There is a strong association of prolactinoma with breast and colorectal cancers diagnosed in their families; iii) 46% of PA patients who were diagnosed with cancer had a strong family history of malignancy, in particular those with NFPA.

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Iron overload impairs the migratory ability of a model of immature and migratory GnRH neurons

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Iron represents a micronutrient for cellular metabolism and aerobic respiration, and is essential for proper brain development in the fetal and early neonatal period. However, excess iron produces toxic build-up via free radical formation. In thalassaemic and juvenile hemochromatosis patients with pubertal failure, iron overload interferes with the correct function of the hypothalamic–pituitary axis, leading to hypogonadotrophic hypogonadism and growth failure, but the mechanisms are still unclear. Aim of this study was to investigate the mechanisms of iron toxicity *in vitro* in GN-11 cells, a model of immature and migratory GnRH neurons. Gene expression analysis by semi-quantitative PCR showed that GN-11 express the iron proteins ferritin (a primary intracellular iron-storage protein) and transferrin (iron-binding extracellular glycoprotein that controls the level of free Fe and deliver iron to cells), as well as hepcidin (an iron regulatory hormone). Exposure of GN11 cells to 150 μM ferric ammonium citrate (FAC) resulted in the inhibition (-35%, $P < 0.05$) of fetal bovine serum (FBS)-induced chemo-migration, assessed by Boyden chamber assay. Pre-treatment with 100 μM deferoxamine, a specific iron chelator, reverted the above reported effect. Time-course experiments showed that 150 μM FAC was associated with induction of phosphorylation of both extracellular signal-regulated kinase (ERK) and 5′ adenosine monophosphate-activated protein kinase (AMPK) after 10 min treatment, as evaluated by Western blotting. Specific ERK and AMPK inhibitors, U0126 and Compound C, respectively, abolished FAC-mediated signaling. Moreover, U0126 and Compound C (both 10 μM) counteracted FAC-driven phosphorylation of acetyl-CoA carboxylase, an AMPK downstream protein. In conclusion, the present data, though preliminary, show that acute iron treatment negatively affects the migration of GN-11 neurons *in vitro*, and is associated with the activatio of ERK and AMPK signaling pathways. We hypothesize that iron overload may impair migration of GnRH neurons from the olfactory placode into forebrain and hypothalamus, where they promote reproductive competence.

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P691

The reproductive endocrine complications of antiepileptic drugs at woman's epilepsy

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Purpose
To study reproductive endocrine complications (REC) of antiepileptic drugs (AEDs) at a woman epilepsy
Materials and methods
One hundred and fifty five women at the age of 16–45 years were included in prospective observational uncontrolled comparative study of the antiepileptic drugs reproductive side effects. Three groups were divided: 1 gr., monotherapy AEDs; 2 gr., polytherapy; 3 gr., no AEDs used. REC lasted more than 6 months were accounted. The diagnosis was established based on comprehensive exam, in accordance with ICD-10. Naranja algorithm was used to determine reliability of communication 'AEDs – REC'.
Results
70 patients (45%) were in 1 gr., 65 (42%) – in 2 gr., 20 (13%) – in 3 gr. 73 patients (47%) were healthy in a total cohort. The overall incidence of REC were 53%, 75% of them due to side effects of AEDs. Comorbid REC was observed in 21 women (13%). In 61 (40%) cases were identified REC due to treatment of AEDs. REC were associated with the taking AEDs in 21 women (30%) at the 1 gr., 40 (57%) patients were healthy. In the 2 gr. REC associated with exposure to AEDs was diagnosed in 38 patients (59%). Only 2 people (10%) retained menstrual irregularities arising from preceding antiepileptic therapy in the three control gr. Differences in the frequency of REC in the comparison groups were statistically significant ($P < 0.001$). Disturbances of a menstrual cycle were determined at 57%, the amenorrhea – 10%, sterility – 10%, a syndrome of polycystic ovaries –7%, dysfunctional uterine bleedings – 3%, a delay of sexual development – 2%, a premenstrual syndrome – 11%. Differences in the frequency of disturbances of a menstrual cycle in the comparison groups were statistically significant above at polytherapy ($P < 0.001$).
Conclusion
Reproductive disturbances are a frequent side effect of antiepileptic drugs at woman epilepsy above at polytherapy. It is necessary monitoring a condition of reproductive health during treatment by antiepileptic drugs.

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P692

DHEA and cortisol response to working memory

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Introduction
Several studies reported DHEA relations to memory and cognition improvement. An anti-cortisol action may contribute to those relations. The effect of working memory (WM) on DHEA levels is unknown. We studied DHEA and cortisol reactivity in humans after a WM stimulus with simultaneous distraction.
Subjects and methods
Twenty eight healthy female volunteers (18–26 years old) were presented a well-established auditory-visual distraction task protocol. Subjects performed one task with working memory (WM) load (WM1) and other without (WM0), 120 min apart, with counterbalanced order across subjects. Each task consisted of 500 trials (15 min), all composed by a task irrelevant sound (80% standard; 20% novel) followed by the visual stimulus that the subject had to classify. Salivary DHEA and cortisol were measured before each task (0 min) and at 30 and 60 min.
Results
DHEA raised after the second task ($P=0.016$), more with WM1 load (190 ± 83 at 0 min vs 290 ± 206 pg/ml at 30 min, $P=0.045$) and cortisol declined after WM0, when it was the first task (1290 pg/ml at 0 min vs 930 pg/ml at 30 min vs 620 pg/ml at 60 min, $P<0.05$) so that DHEA and cortisol levels were higher when WM1 was the second task (DHEA: $P=0.022$, cortisol: $P=0.004$). Cortisol:DHEA ratio was lower in the second task ($P=0.001$) and decreased after both tasks performance ($P=0.036$).
Discussion
Under distraction, higher DHEA levels were found after the performance of two consecutive cognitive tasks. DHEA raised and cortisol did not decline after WM load when it was the second task, suggesting that consecutive cognitive tasks and WM load may raise DHEA and prevent cortisol decrease. DHEA increase with WM load contributed to lower cortisol:DHEA ratio after that task. Previous studies related higher DHEA levels with memory enhancement. The present results suggest that cognitive tasks and WM load may also be a stimulus against DHEA decay.

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Neuroprotective effects of estrogen rely on neuroglobin upregulation

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Estrogens, in particular 17β-estradiol (E_2), orchestrate cellular mechanisms involved in the development and differentiation of various neuron populations, modulation of synaptic plasticity, induction of neuronal survival, and neurogenesis. Beneficial effects of E_2 have been widely reported against a variety of insults, including H_2O_2 injury, serum deprivation, and glutamate excitotoxicity. Recently, we identified E_2 as an endogenous modulator of a new neuroprotectant protein, neuroglobin (Ngb). Here, our principal aim is to identify the role played by E_2-induced Ngb up-regulation in neuroprotective signaling pathways. In neuroblastoma SK-N-BE cells Ngb is expressed in the nucleus, mitochondria and is scattered in the cytoplasm. Upon E_2 stimulation, Ngb reallocates mainly into mitochondria where the physical association with the mitochondrial cytochrome *c* occurs. E_2 pretreatment before H_2O_2 addition strongly enhances Ngb:cytochrome *c* association reducing its release into the cytosol. As a consequence, a decrease of caspase-3 activation and, in turn, of the apoptotic cascade activation takes place. E_2 induces Ngb level regulation also in astrocytes, where this globin is required for E_2 effects in preventing lipopolysaccharide (LPS)-induced cytochine release. All these effects are mediated by estrogen receptor β (ERβ) via genomic and extranuclear signals involving p38/MAPK pathway. As a whole, the well known neuroprotective effects elicited by E_2 may, at least in part, be explained by an enhanced Ngb expression in neurons and astrocytes. The principal role played by Ngb in the brain could be related to the reduction of neuronal death by resetting the trigger level of apoptosis and inhibition of pro-inflammatory cytokines expression, leading to the onset of physiological response to stress. E_2 acts to accelerate Ngb neuroprotective effect rapidly enhancing its levels in both neurons and astrocytes.

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Diagnosis and management of patients presenting hyponatremia while receiving parenteral nutrition

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Introduction
Hyponatremia is the most frequent electrolyte alteration in hospitalized patients, and is associated with increased morbimortality. Hyponatremia in patients receiving parenteral nutrition (PN) is generally overlooked. Our objective was to classify the types of hyponatremia observed in patients on PN, and to describe the prescribed treatments and evolution.
Material and methods
We undertook a prospective study of all patients on medical wards receiving PN with hyponatremia – serum sodium (SNa) <135 mmol/l – attended by our team between 01/06/12 and 01/12/12. Study included physical examination, serum (S) /plasma (P) and urine(U) electrolytes and osmolality (Osm), glycemia, circulating TSH, fT_4, cortisol, ACTH, urea/creatinina, and transaminase levels.
Results
Twenty out of 85 patients (23.5%) presented hyponatremia (9 women), average age 68.5 (s.d. 13.15), SNa 130.8 mmol/l (s.d. 4.03), nadir SNa 128.95 (s.d. 3.2). 70% (14) were euvolemic, 20% (4) hypovolemic, and 10% (2) hypervolemic. SNa euvolemics: 130.5 (IR 129–133), P_{osm} 274 mOsm/Kg (IR 266.7–280). U_{Na}: 121 mmol/l (IR 82–128), U_{osm}: 454 mOsm/Kg (IR 366–786).
Diagnosis/treatment: euvolemics
Three cases of pain-related physiologic ADH elevation were treated with analgesics: SNa pre-treatment 132.5 (IR 131.6–137.6) and post-treatment 137.5 (IR 133–138). 10 cases presented SIADH. Of whom 8 received fluid restriction and increased sodium in PN: SNa pre-treatment 129 (IR 129–133) post-treatment 134.0 (IR 133.2–136.1), 2 required tolvaptan: SNa pre-treatment 127.83 (IR 126.6–129) post-treatment 137 (IR 136–138); In one case of thiazide-induced hyponatremia the diuretic was discontinued: SNa pre-withdrawal 133, post-withdrawal 137. Hypovolemics were treated with an increase in PN volume. SNa pre-treatment 132 (IR 124.2–133.7), post-treatment 134 (IR 132.1–136.5). HYPERVOLEMICS received furosemide and NP volume was reduced: SNa pre-treatment 130.50 (IR 130.0–131.0), post-treatment 137.5 (IR 133–142).
Conclusions
SIADH was the most frequent cause of hyponatremia in our PN-treated patients. A correct diagnosis of the cause of hyponatremia in patients receiving PN is essential for adequate treatment of this common electrolyte disorder.

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P695

Des-acyl ghrelin acts directly and independently and counteracts acyl-ghrelin-induced neuronal activity in the central melanocortin system of rats

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Ghrelin, the endogenous GH secretagogue, has an important role in metabolic homeostasis. It exists in two major molecular forms: acylated (AG) and des-acylated (DAG). Many studies suggest different roles for these two forms in energy balance regulation. In the present study, we compared the effects of acute intracerebroventricular (ICV) administration of AG, DAG and their combination (AG+DAG) to young adult Wistar rats on food intake and central melanocortin system modulation. The results showed that ICV DAG did not affect food intake when compared to saline-treated rats. The same treatment significantly increased the number of c-fos (marker of neuronal activity) positive neurons in the arcuate (ARC), paraventricular (PVN) and solitary tract (NTS) nuclei, while DAG suppressed AG-induced neuronal activity in PVN and NTS, 2 h postinjection. Central DAG increased melanocortin-4 (MC4R) and decreased melanocortin-3 receptor (MC3R), agouti-related protein (AgRP) and proopiomelanocortin (POMC) hypothalamic gene expressions, 5 h postinjection. These results demonstrate that DAG acts directly, centrally and AG-independently to increase neuronal activity in the melanocortin system in rats and is able to counteract AG

actions in hypothalamic PVN and NTS in the brainstem. Together with the absence of the effect on energy intake, these results indicate that DAG could affect energy homeostasis by modulation of energy expenditure via central melanocortin system.

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P696

Evaluation of the efficacy of midnight salivary cortisol plus dexamethasone suppression test as screening for the diagnosis of hypercortisolism within an at-risk population

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Introduction
Endogenous Cushing's syndrome (CS) is a rare, infradiagnosed and severe disease that carries high morbidity and mortality. Delays in treatment initiation reduce reversibility of symptoms and increase mortality rate. Current gold standard for screening, 24 h urinary free cortisol, has a low reproducibility and specificity, and sampling is complicated. Midnight salivary cortisol (MSVC) determination is considered an easy and cheap test. We intend to assess its feasibility combined with suppression test to screen CS. This initiative has been developed by the Cushing's disease (CD) working group, member of the Sociedad Española de Endocrinología y Nutrición (SEEN) and supported by Novartis.
Methods/design
Epidemiological cross-sectional study to asses MSVC plus suppression test. Secondary objectives are to describe the prevalence of CS and CD in the studied population. Patients will be performed the two tests, results from both, clinical characteristics and final diagnosis of CS or CD will be recorded.
Results
This project, named CRIbado en SALIva De Alteraciones del cortisol (CRISÁLIDA) was initiated on November 2012, with 13 participating sites accross Spain. Sample size will be 609 patients and results from an interim analysis are expected on April 2013. Study population includes a group of patients considered at risk of presenting high cortisol levels, presenting at least 2 of the following symptoms: obesity (BMI >30), hard to control hypertension (SBP >140 and DBP >90 mmHg, >2 drugs), poorly controlled diabetes (HbA1c $>7.0\%$), severe osteoporosis (-2.5 S.D. in *T*-score) or virilization syndrome. Exclusion criteria: pregnancy, age >70 years, glucocorticoid treatment, CS or CD diagnosis, chronic renal impairment or hepatopathy, between others.
Conclusion
CRISÁLIDA is a multicentric project designed to identify most relevant characteristics of an at-risk population and to move forward in the screening and diagnosis of a rare disease (CD).

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Impact of long acting somatostatin analogs vs successful surgery on glucose metabolism in Cretan acromegalic patients

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Introduction
Acromegaly is associated with increased cardiovascular morbidity and mortality, in part, as a consequence of the increased insulin resistance that frequently accompanies GH hypersecretion. Glucose tolerance may worsen in patients treated with somatostatin analogs (SSA).
Objective
To compare the long term effects of SSA treatment to successful surgery on glucose metabolism in Cretan acromegalic patients.
Design and methods
This is a cross-sectional study. We studied 22 normoglycemic acromegalics (12 female/10 male, age 53 ± 14). Fifteen patients were on SSA treatment (seven were controlled) and seven patients were surgically cured. The median period of SSA treatment was 10 years. Twenty seven age-and sex-matched healthy subjects were studied as controls. Glucose homeostasis was studied using the indexes stated below, at baseline and following an oral glucose challenge (OGTT 75 g). The areas under the response curves of glucose (AUC_{GLUC}) and insulin (AUC_{INS}) during OGTT were estimated.
Results
On average, both groups of acromegalic patients had significantly elevated fasting glucose, compared to controls ($P_{surg}=0.01$, $P_{ssa}<0.001$). AUC_{GLUC} after OGTT was significantly elevated in all SSA-treated patients ($P_{ssa\ controlled}=0.04$, $P_{ssa\ not\ controlled}=0.003$). AUC_{INS} was significantly decreased only in controlled SSA-treated acromegalics ($P=0.035$). No significant differences in AUC_{GLUC} or AUC_{INS} were observed between surgically cured acromegalics and the control group. Basal insulin secretion evaluated by HOMA-B%, was significantly decreased in all SSA-treated patients compared to controls ($P=0.001$) but no difference was observed in basal and OGTT-derived insulin resistance evaluated by HOMA-IR, QUICKI and ISIComposite between acromegalics and controls.
Conclusion
Cretan acromegalics, even if cured had elevated fasting plasma glucose. SSA treatment resulted in higher glucose values in the fasting state and after OGTT, because of impaired insulin secretion, but had no impact on insulin resistance.

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Prevalence and causes of hyponatremia in patients with endocrine disorders

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Objectives
To study the prevalence and causes of hyponatremia in patients with various endocrine diseases.
Methods
We had retrospectively analyzed the results of biochemical laboratory studies performed during one calendar month at the tertiary care clinic for endocrinology patients for the variations in the serum sodium concentrations. Blood sodium levels <135 mmol/l were considered as hyponatremia. For all patients with hyponatriemia addition data including demographics, clinical diagnosis of primary and comorbid conditions, physical and instrumental examination records, duration of hospitalization were accessed through electronic patient's chart.
Results
During this period a total of 1511 studies of serum sodium were performed, including hyponatremia was observed in 1.0% of patients (15/1511, 3 men/12 women). Hyponatremia occurred four times more frequently in hospitalized patients than those undergoing outpatient examination/treatment – 0.8 vs 0.2% respectively. The average age of patients with hyponatremia was 48.6 years (0.75, 85). Length of hospital stay among patients with hyponatremia on average was 26.5 days, with two patients were observed in the out-patient conditions, length of stay remaining patients ranged from 6 to 116 days. Development of hyponatremia was multifactorial and the causes included: use of diuretics – 46.7% of cases, exacerbation of adrenal insufficiency – 40%, nephropathy in type 2 diabetes mellitus – 20%, redistributional hyponatremia in decompensated type 1 diabetes mellitus due to hyperglycaemia – 6.7%, loss of sodium through the post-operative drainage from the abdominal cavity – 6.7%, acute urinary infection – 6.7%.
Conclusion
The overall prevalence of hyponatremia in patients with endocrine disorders is about 1%. Pathogenesis of hyponatremia in most cases multifactorial and most often associated with the use of diuretics, presence hypocorticism and renal disease.

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P699

Acute exposure of Bisphenol-A from electronic gadgets does not induce oxidative stress in the rat's brain

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Objective
To investigate the effects of BPA on oxidative damage in terms of activity level of antioxidant enzymes in different regions of the rat brain.

Background
The ever increasing uses of electronic gadgets are becoming a widespread source of Bisphenol-A accumulation. As studies have been reported that low level BPA accumulation may produce neurological effects but still limited studies have re-examined for its adverse effects in terms of acute exposure from electronic devices.
Methodology
In this study, BPA migration was estimated through physio-chemical parameters and leachate (equivalent to 4 mg/kg body weight) was used for animal dosing. Three groups of Albino Wister rats (190±20 g) were used for control, sham, and treated. The antioxidant enzymes including superoxide dismutase (Mn-SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione level (GSH) were measured in different brain regions, i.e. corpus striatum, frontal cortex, thalamus and midbrain.
Results
No significant changes were observed in most of the brain regions yet the level of GPx activity in corpus striatum (29.65±0.98 nmol/min per mg protein) and level of GSH activity in frontal cortex (2.33±0.12 μmol/g protein) was found to decrease significantly ($P<0.05$) when compared to controls. In addition, no significant effects were observed for the oxidative damage in brain regions of sham group when compared to control group.
Conclusion
Thus study suggests that acute exposure (4 mg/kg body weight per day up to 28 days) of BPA does not induce significant oxidative damage in the rat's brain. Furthermore, study might re-examine before affirm the final remark for subscribers and regulatory bodies at similar doses.

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P700

Oxytocin modulates olfaction-related behaviour and essential for onset of sexual behaviour in both sexes of mice

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In microsmatic animals, the olfactory cues initiate wide variety of neuroendocrine and behavioural responses. These behaviours are under the influence of sex hormones however some neuropeptide plays pivotal role. Oxytocin is involved in various social behaviours in mammalian species. However, oxytocin-deficient (OTKO) mice successfully accomplish reproduction although females cannot rear their pups because of lack of lactation. Though oxytocin is not essential for sexual behaviour, it is still possible to have some role in mating interaction. In this study, we employed alternate choice paradigm to assess odour preference to investigate the sociosexual behaviour in two experimental paradigms; social approaching to sexual partner odour evaluated by an alternate choice paradigm, and direct social interaction in the semi-natural environment using OTKO mice of both sexes to determine the functional significance of oxytocin. In preference test, experimental mice were given a choice of two airborne odours derived from active male and estrus female, or active male and castrated male. The odours were presented by airflow through air-inlets on walls of the preference test apparatus, and time spent nose-poking into the inlet was recorded for 5 min to determine olfactory preference. Wild-type mice significantly preferred sexually active opposite-sex odour to the others, whereas OTKO mice showed vigorous but indifferent nose-poking to any odours. Following odour preference, the OTKO and wild-type mice were placed with an opposite-sex individual in the semi-natural environment, and social interactions were tested. OTKO mice required significantly longer time to accomplish sexual behaviour. We concluded that oxytocin plays an important role in regulation of sexual behaviour, especially in a component of olfaction-related behaviour, in both male and female mice.

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P701

Obestatin is associated to muscle strength, functional capacity and cognitive status in old women

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Introduction
Obestatin has been proposed to have anorexigenic and anti-ghrelin action. An association has been found between ghrelin and its decline with ageing and muscle strength and functional capacity, probably mediated by GH–IGF1 axis. However, no information is available in relation to the role of obestatin in muscle strength and functional capacity.
Objective
The objective was to study obestatin concentrations in relation to handgrip strength, functional capacity and cognitive state in old women.
Methods/design
Prospective study; 110 women (age 76.93±6.32 years) from the Mataró Ageing Study were included. Individuals were characterized by anthropometric variables, grip strength, Barthel and assessment of cognitive impairment (MiniCognoscitive Examination (MCE), Spanish version), depressive status by the geriatric depression scale (GDS) and frailty by Fried criteria. Obestatin was measured by IRMA.
Results
Obestatin was negatively correlated to handgrip at basal time-point ($r=-0.220$, $P=0.023$) and at two years follow-up ($r=-0.344$, $P=0.002$). Obestatin divided into quartiles, showed a negative linear association with handgrip: 11.03±4.88 kg in 1st quartile, 8.75±4.08 kg in 2nd, 8.11±3.66 kg in 3rd and 7.61±4.08 kg in 4th quartile ($P=0.018$). Higher obestatin levels were associated to weakness (categorized by handgrip of frailty criteria): 2.24±0.42 ng/ml in weak vs 1.87±0.57 ng/ml in non-weak; $P=0.01$). Relative decrease in MCE at two year follow-up was significantly higher in individuals in the 4th quartile of obestatin in comparison with individuals in the 1st quartile (7.25±12.03% in 4th quartile vs −0.09±10.48% in 1st quartile; $P=0.046$). In addition, relative decrease in Barthel score was significantly higher in individuals in the 4th quartile of obestatin in comparison with individuals in the 1st quartile (3.77±5.77% in 4th quartile vs 0.46±3.19% in 1st quartile; $P=0.019$).
Conclusions
Obestatin is associated to low muscle strength, impaired functional and cognitive capacity in old women participating in the Mataró Ageing Study.

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P702

Copeptin for subtype differentiation of abnormal vasopressin release in SIADH: reclassification and characterization of a novel subtype

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Introduction
The syndrome of inappropriate antidiuresis (SIADH) is the most common cause of hypoosmolality. Ectopic AVP hypersecretion has long been considered as the primaryn mechanism of SIADH. But different types of osmoregulatory defect in AVP release have been described in this disorder. A comprehensive characterization of these different forms of SIADH in a large cohort of patients may provide important new insights into the still incompletely understood pathophysiology of SIADH, as well as the variable need for therapeutic management in SIADH.
Methods and design
Differences in pathological AVP osmoregulation in SIADH were characterized by serial measurement of plasma copeptin, a reliable AVP surrogate marker, in 50 patients with SIADH during osmotic stimulation. The physiological relationship between plasma copeptin and osmolality was defined by means of 68 healthy controls, who underwent the same protocol of osmotic stimulation.
Results
In healthy subjects, a close correlation was found between plasma copeptin and osmolality with an osmotic-threshold of 282±4.3 mOsm/kg H_2O. In SIADH, five different types of defective AVP osmoregulation were found: 10% of patients showed a markedly elevated and osmotically-independent form of copeptin release (type A); 14% demonstrated a linear osmotic response to rising serum osmolality, but with an abnormally low osmotic-threshold (type B); 44% revealed a fixed, plateau-like copeptin secretion (type C); and 12% of patients had an AVP-independent SIADH with undetectable copeptin levels. A different, new type of SIADH was found in 20% of patients, demonstrating an inverse relation of decreasing plasma copeptin levels to increasing osmolality (type E).

Conclusion
These findings confirm the concept of ectopic AVP secretion (type A) and AVP-independent antidiuresis (type D) as osmodefective subtypes of SIADH. But the main pathophysiological alteration in most patients with SIADH was the profound impairment of AVP osmoregulation well into the normoosmotic range. Moreover, firstly a novel subtype of SIADH could be described, presumably related to impaired nonosmotic inhibitory pathways in combination with altered osmoregulatory function.

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P703

Neurodegenerative and inflammatory biomarkers in cerebrospinal fluid in patients with Cushing's syndrome

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Background
Patients with Cushing's syndrome (CS) in long-term remission have impaired cognitive function. Cerebrospinal fluid (CSF) biomarkers are important diagnostic tools in the work-up of patients with cognitive impairment. The aim of this study was to analyze biological markers in CSF from patients with CS in remission. Owing to the overlapping similarities between patients with CS and dementia, i.e. cognitive dysfunction and hypercortisolemia, the main hypothesis was that the pattern of CSF biomarkers in CS patients resembles the pattern found in other neurodegenerative disorders.
Patients and methods
This was a cross-sectional, case-controlled, single centre study. Twelve women previously treated for CS, six matched controls (age and educational level) and three women with active CS were studied. The following neurodegenerative CSF markers; total τ, hyperphosphorylated τ, amyloid β peptides (Aβ1–42, Aβ38, Aβ40 and Aβ42), soluble amyloid precursor protein α and β, neurofilament light proteins, glial fibrillary acidic protein and monocyte chemoattractant protein 1, and inflammatory CSF markers; interferon-gamma, interleukin β (ILβ), IL2, IL4, IL5, IL8, IL10, IL12p70, IL13 and tumour necrosis factor α were analysed.
Results
The mean age (mean ± S.D.) was similar in patients with CS in remission (44.9 ± 14 years) and controls (42.3 ± 15.7 years; $P = 0.726$). No differences were seen in concentrations of any neurodegenerative biomarkers between either patients with CS in remission and controls or between patients with active CS and controls. Nor was the concentration of inflammatory biomarkers different between the groups, except for IL8 which was significantly higher in patients with active CS compared to controls.
Conclusions
The pattern of neurodegenerative and inflammatory biomarkers in CSF from patients with CS does not differ from healthy controls. The underlying mechanisms of the cognitive deficits in CS are different from those seen in neurodegenerative disorders and remains to be explained.

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P704

Evaluation of GH deficiency and central adrenal insufficiency in patients following craniospinal irradiation in young ages: comparison between the glucagon stimulation test and the insulin tolerance test

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The patients following craniospinal irradiation (CSI) in young ages due to posterior fossa tumors had high risk of hypopituitarism. The glucagon stimulation test (GST) may be used as alternative the insulin tolerance test (ITT) in the diagnosis of GH deficiency (GHD) and central adrenal insufficiency (CAI) when insulin-induced hypoglycaemia is contraindicated e.g. history of convulsions, coronary heart disease, also it can be carry out as an outpatient. The aim of this study was to compare the GST and ITT for diagnostic GDH and CAI in patients following craniospinal irradiation (CSI) in young ages.

Methods
Thirteen patients were examined (five girls, eight boys, median age, 19 years (17; 22)). All had posterior fossa tumors in history. Median age at the time of treatment was 13 years (8; 14), Median follow-up was 5 years (3; 6). All patients received resection of the tumor, chemotherapy and CSI (35 Gy for whole brain, 55 Gy for tumor area and 32 Gy for spine). All of them performed ITT and GST. Severe GHD was diagnosed when GH was <3 ng/ml, cortisol cut-off for normal response was level 550 nmol/l.
Results
9/13 patients diagnosed severe GHD (median GH, 0.89 ng/ml (0.6–1.4)) in GST and 12/13 patients revealed GHD (median GH, 0.85 ng/ml (0.37–1.65)) in the ITT. 9/13 patients had CAI in GST (median peak plasma cortisol, 385 nmol/l (335; 476)). But the diagnosis was refuted in 4/9 patients in ITT, peak plasma cortisol in this was >550 nmol/ml (Me 645 nmol/l (624–676)).
Conclusions
The obtained results allow us to recommend GST as initial outpatient test. If patient have peak GH <3 ng/ml and peak cortisol <550 nmol/ml in GST, ITT is required. Because the sample is small GST can't substitute ITT now. The study continues.

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P705

Diurnal melatonin profile in patients with acromegaly

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Introduction
Cardiovascular complications are the most relevant cause of mortality in patients with acromegaly.
Aims
To evaluate the association of diurnal melatonin profile with arterial hypertension in acromegalic patients.
Subjects and methods
Thirty seven patients (15 men and 22 women; aged 18–75 years) with acromegaly were under investigation. Blood samples for GH and, IGF1 were taken in fasting state. 6-Sulfatoxymelatonin (6-SMT), the main melatonin metabolite, was determined by fluorometric assay by C. Druex in two separated urine portions: in the daytime (6-SMTd) and nighttime (6-SMTn). Routine measurements of blood pressure (BP) were done. The average of three measurements with a mercury sphygmomanometer was used in all analysis. The BP varied in large SBP (80–176 mm/Hg); DBP (58–116 mm/Hg). Arterial hypertension (AH) was diagnosed when BP was above 140/90 mm/Hg. Disease activity was evaluated according to the Consensus Conference criteria (2000). Data are given as mean ± S.E.M. and nonlinear regression model equations parameters.
Results
In patients with somatotropinoma (GH, 22.8 ± 3.5 ng/ml) and somatomamotropinoma (GH, 26.3 ± 5.3 ng/ml) the diurnal level of 6-SMT (96.5 ± 9.8 nmol) including 6-SMTn (46.8 ± 5.6 nmol) and 6-SMTd (49.7 ± 5.6 nmol), 6-SMTd/6-SMTn (1.46 ± 0.22) have been found out. It was revealed that in patient without AH the modulating action of melatonin on DBP is approximated by equations: $DBP \approx 48.2 + 7.2 \times \ln$ (6-SMTn) ($R^2 = 37.9\%$; $r = 0.61$; $P = 0.02$). In patient with AH the negative associations exist between level of 6-SMTn and SBP ($R^2 = 27.8\%$; $r = -0.52$; $P = 0.01$), and 6-SMTn and DBP ($R^2 = 47.8\%$; $r = -0.69$; $P = 0.004$). The 90% of patient with AH have the diurnal level of 6-SMT <120 nmol including 6-SMTn <80 nmol and 6-SMTd <40 nmol.
Conclusion
In patients with active acromegaly AH is associated with 6-SMT <120 nmol and 6-SMTd/6-SMTn >1. For hypertensive patients with acromegaly decreased level of melatonin in nighttime is more appropriate than in daytime. Altered diurnal profile of melatonin may be one of the cause of AH in patients with acromegaly.

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P706

Comparative analysis of the hormonal, MRI and morphological characteristics of patients with Cushing's disease remission and no remission after neurosurgical treatment

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Introduction
Long term remission of CD is achieved in 65–83% after neurosurgery, while the remaining cases have recurrence or continued tumor growth. This paper deals with the problem of CD prognostic factors after neurosurgery.
Design
ACTH and cortisol blood levels, free cortisol in daily urine, MRI, histology (hemotaxiline–eosine staining) and immunohistochemistry (with antibodies to the pituitary tropic hormones, Ki-67, CD31 and VEGF) of the removed corticotropinomas tissue were compared between two groups of patients with CD (totally 46): 31 (67.4%) patients in remission (first group) and 15 patients (32.6%) without CD remission (second group) after neurosurgery, who were observed on the average for 2 years.
Results
ACTH and cortisol blood levels didn't differ between two groups of patients before neurosurgical treatment ($P=0.9$ and $P=0.8$ respectively). The content of free cortisol in the daily urine before treatment was significantly higher in patients from the second group ($P=0.0006$). Volume and topography of corticotropinomas on MRI also didn't differ in patients from both groups ($P=0.8$ and $P=0.9$ respectively). After pituitary adenomectomy in 30 (96.8%) patients from the first group developed clinical and laboratorial signs of adrenal insufficiency, whereas only 5 (10.9%) patients from the second group were diagnosed with adrenal insufficiency. Histologically the incidence of basophilic-cell tumors ($P=0.4$), mitosis ($P=0.9$), necrosis ($P=0.5$), hemorrhage ($P=0.7$), stromal edema ($P=0.9$), angiomatosis ($P=0.6$) and anterior pituitary hyperplasia ($P=0.5$) didn't differ in the corticotropinomas of patients from the both groups. Significant difference of frequency of mono-($P=0.8$), bi-($P=0.8$), and polihormonal ($P=0.4$) adenomas in patients from the both groups is not found. Labeling index of Ki-67 in corticotropinomas in patients without CD remission was significantly higher than in adenomas of patients with remission ($P=0.02$). Significant differences of CD31 ($P=0.3$) and VEGF ($P=0.7$) expression in the tumors of patients from both groups did not find.
Conclusion
Such factors as the level of free cortisol in the daily urine, the absence of adrenal insufficiency after neurosurgery, increased labeling index of Ki-67 may be considered as poor prognostic factors of CD after neurosurgery.

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P707

Prevalence and clinical significance of hook-effect and macroprolactinaemia phenomenons in patients with prolactinomas

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Introduction.
Circulating prolactin is predominantly monomeric, but when there presents more than 60% of polymeric, less bioactive forms, phenomenon of macroprolactinemia is diagnosed.
For prolactinomas, serum prolactin levels generally parallel to tumor size, but tumor mass and prolactin levels may be dissociated due to hook-effect, when falsely low prolactin level observed. It is excluded by serial dilutions of serum samples.
The frequency and clinical significance of that phenomenons are still less understood.
Objective
To assess the prevalence and clinical significance of Hook-effect and macroprolactinaemia phenomenons in patients with prolactinomas.
Materials and methods
190 patients with prolactinomas (87% females and 13% males) were examined with conventional laboratory and instrumental methods. Pituitary tumors were classified according to size as microadenomas – <10 mm, mesoadenomas – from 10 to 20 mm, macroadenomas – from 20 to 40 mm, giant – >40 mm in diameter.
Results:
Macroprolactin was revealed in 65 cases (34%) of asymptomatic hyperprolactinemia. Phenomenon of macroprolactinaemia was found in 5% of patients with microadenomas, and was negative in patients with other tumor sizes ($P<0.01$). Average prolactin content in those patients was 1550 ± 1.7 mU/l. Average macroprolactin content was $64.5\pm4\%$.
Hook-effect phenomenon was observed in 3 patients with macroadenomas and marked in elevation of prolactin level from 1500 to 22 390 mU/l, from 3180 to 130 785 mU/l, from 4200 to 240 168 mU/l after dilution. In patients with other tumor size this phenomenon was negative ($P<0.01$).
Conclusion
As phenomenons of macroprolactinaemia and hook-effect cannot be reliably distinguished on clinical criteria alone, we recommend routine screening for macroprolactin in patients with asymptomatic microprolactinomas and hook-effect exclusion by serum dilution in patients with macroadenomas in oder to avoid misdiagnosis and mismanagement.

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P708

Hyponatremia in the emergency room: characteristics, and initial diagnostic approach

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Introduction
Hyponatremia is common in the emergency room, albeit frequently overlooked. Our objective was to describe the characteristics of hyponatremia in a cohort of emergency room patients, and evaluate how hyponatremia was studied and followed up.
Material and methods
We studied all 211 patients under 70 years-of-age who presented/developed non-translocational hyponatremia (serum sodium (SNa) <135 mmol/l) during the first 48 h of their stay at the emergency room of our general hospital in August 2012. Patients are classified by physical examination and the presence of hemodilution or hemoconcentration (urea/creatinine, hematocrit).
Results
Average age was 47 (s.d. 16), 54.5% (115) were women. 31% (66) had presented hyponatremia previously. 8.5% (18) were hypovolemic, 3.3% (7) hypervolemic, 62.6% (132) euvolemic (64.8% of whom experienced pain, 40.8% nausea, 29% both). 25.6% (54) lacked sufficient data for classification. Mean initial SNa: 132.3 mmol/l (s.d. 3.35), Mean nadir SNa: 131.6 mmol/l (s.d. 3). 5.6% (12) presented polydipsia. 31.8% (67) were medicated with selective serotonin reuptake inhibitors, 6.6% (14) thiazides, 6.6% (14) opiates, 7.6% (16) benzodiazepines, 6.6% (14) antiepileptics, 2.8% (6) antipsychotics. Only 7 (3.3%) patients had osmolality (plasma/urine) determined, 13 (6.2%) urine electrolytes. 21% had TSH levels. Only two patients had cortisolemia measured. A comprehensive diagnostic study of hyponatremia (physical exploration and laboratory tests) was undertaken only by Endocrinologists. In 34% (72) SNa levels improved within 24 h, in 17.5% (37) they dropped. In the rest SNa remained stable or was not repeated. 2.8% (6) developed overcorrection of SNa. None were relowered. The most common principal diagnoses were urinary tract infection: 10.5% (22), neoplasia: 8.5% (18) – a third of whom had ENT cancer – and gastroenteritis: 7.6% (16). In 1.4% (3) hyponatremia was the principal diagnosis and in 15.2% (32) a codified diagnosis. After discharge, 36% (27) of the 75 patients with follow-up data remained hyponatremic.
Conclusions
In a majority of cases, hyponatremia was inadequately studied in the emergency room of our hospital, hindering a correct diagnosis and treatment of this important disorder.

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P709

Modulation of Na^+/K^+-ATPase activity of rat brain synaptosome by norepinepherine and serotonin

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Sleep and wakefulness are behavioral and physiological activities. It is a modified form of the basic rest activity cycle. Humans usually fall asleep by entering in non Rapid Eye movement sleep, a phase accompanied by characteristic changes in

the encephalogram (EEG). The person next moves to REM sleep, which is characterized not only by rapid eye movements but also by inhibition of skeletal muscle tone. These two states alternate with each other during sleep cycle. It has been found that REM sleep is generated as a result of excitation of Cholinergic PS on neurons and inhibition of monaminergic PS off neurons. Moreover the REM sleep deprivation induced increase in Na^+/K^+-ATPase activity which is partially mediated by NE. Serotonin has been found to increase during REM sleep. These facts implicate that both serotonin and norepinepherine are involved during REM sleep. This study has been initiated to find out the effect of norepinepherine and serotonin and the blockers prazosin and propranolol in different permutations and combinations on Na^+/K^+-ATPase activity. Brain from the male wistar rats weighing 250–280 g was extracted and subjected to homogenization, synaptosome was prepared and Na–K-ATPase activity was estimated under the influence of NE (100 μM), 5HT (μM), prazosin (5 mM) and propranolol(5 mM) in different combinations. Both NE and 5HT increase the Na^+/K^+-ATPase activity individually and also synergistically when used in combination but in presence of receptor antagonists a decrease is observed. Moreover prazosin and propranolol also decrease the basal values of Na^+/K^+-ATPase activity. We conclude that results will help in exploring the therapeutic possibilities for neurological and other movement disorders associated with norepinepherine and serotonin.

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P710

Adulthood germ cell tumor: a case report

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Introduction
Germ cell tumors (GCTs) are classified as extragonadal if there is no evidence of a primary tumor in the gonads. They typically occur in the midline structures. Newly diagnosed adult cases of pituitary or pineal glands germinomas are very rare since most of the cases are diagnosed in the mid teens, presenting 14:1, on behalf of the male gender. The estimated incidence of this tumor in western countries is between 0.4–3.4%, being more frequent in the Asia.
Case report
A 30-year-old man, was referred to Endocrinology in August 2007, complaining of lack of energy, asthenia, decreased body hair, decreased libido and erectile dysfunction, with no ejaculate, since December 2005. He was previously observed by Urology, carried out thyroid function and was medicated with levothyroxine 100 μg/day since March 2007. Decreased hairiness (axillary, trunk and beard) and decreased testicular volume, without other major changes on physical exam. In October 2007, starts complaints of headaches and blurred left vision. The patient was observed by Ophthalmology, which diagnosed bitemporal hemianopsia, and performed pituitary MRI which revealed a bulky suprasellar lesion with $16\times23\times20$ mm. The endocrinological evaluation revealed hypopituitarism. He underwent pituitary surgery at May 2008, with near total resection, because of proximity to the optic chiasm. The histological exam revealed germ cell tumor. According to the radiosensitive of these tumors treatment was completed with external radiotherapy. After surgery, the hormonal evaluation showed panhypopituitarism and diabetes insipidus. The patient remains clinically stable, on hormonal replacement therapy and without evidence of recurrence.
Discussion
Owing to its rarity in adulthood, not only by location but also to its multifaceted clinical presentation, requires multidisciplinary assessment. The cranial location requires surgical treatment which is often near total resection, because of risk of surgical complications. Therefore, radiation therapy is usually necessary. Intracranial germinomas have a reported 90% five years survival, so follow-up should be extensive.

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P711

Infrasellar gangliocytoma causing cushing's disease: a case report

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Gangliocytomas are uncommon neuronal tumors that can sometimes cause endocrine syndromes. A few such cases have been reported in association with Cushing's disease. In most instances, the tumor contained CRH and was associated with pituitary corticotroph hyperplasia or adenoma (mixed lesion). Only one case of isolated gangliocytoma causing Cushing's disease per se has been described.
We report the case of a 62 year-old woman whose clinical picture and endocrine testing clearly demonstrated ACTH-dependent Cushing's syndrome. Pituitary MRI showed a 12 mm homogeneous, T2-weighed hyperintense, infrasellar mass with delayed contrast enhancement. The mass was bulging into the sphenoidal sinus with scalloping of the clivus. Transsphenoidal surgery was performed and allowed complete resection of the tumor with sparing of the whole normal anterior pituitary. Very low postoperative serum cortisol and ACTH levels were observed, both in the early postoperative period and one month later, proving that the resected lesion was entirely responsible for the clinical picture. Pathology and immunohistochemistry demonstrated a benign tumor composed of mature neuronal cells most likely arising from the posterior pituitary, resembling hypothalamic ganglion cells and expressing ACTH. The gangliocytoma was surrounded by a rim of pituitary tissue containing some ACTH-producing endocrine cells. Careful analysis of the resected lesion did not reveal any pituitary microadenoma. CRH immunostaining of the tissue is currently being performed in order to determine whether the gangliocytoma was also responsible for an associated CRH-induced corticotroph hyperplasia.
This infrasellar ACTH-containing gangliocytoma singles out the few reported cases of gangliocytomas causing Cushing's disease by CRH-induced corticotroph hyperplasia or adenoma. Further analyses on the resected tissue will hopefully allow us to better describe the case of our patient and contribute to the understanding of this rare entity.

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P712

Effects of GH therapy in carbohydrate metabolism in spanish adults with GH deficiency

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GH deficiency (GHD) in adults is characterized by a tendency towards fat mass gain and may predispose to type 2 diabetes mellitus.GH replacement (GHR) is associated with impaired insulin sensitivity shortly after starting therapy, reflected by increased fasting glucose and insulin levels. Available evidence suggests that concerns regarding glucose intolerance in patients receiving long-term GHR have not been substantiated. However, several environmental and lifestyle-related factors could influence glucose abnormalities in patients with GHD, and no study has specifically addressed this issue in spanish patients. Thus, we aimed to describe the evolution of carbohydrate metabolism (fasting glucose (FG) and HbA1c) and ascertain possible risk factors for developing glucose abnormalities in adult patients receiving GHR.
We analyzed retrospectively 34 GDH adults (mean age 40.4 years; 16 females) from our centre who received GHR for at least 2 years (mean duration of treatment was 7.4 years). FG, HbA1c and anthropometric parameters were measured before starting treatment and at the end of the follow-up. Associations were tested by Mann–Whitney *U* test between baseline variables (age, BMI, total body fat, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, mean maintenance dose of GH, and glucocorticoid replacement) and these metabolic variables.
None were diabetic at baseline. FG and HbA1c were significantly higher at the end of follow-up when compared to baseline ($+6.7$ (s.d.11.08) mg/dl $P=0.001$ and $+0.2$ (s.d.0.4) % $P=0.014$ respectively). Average HbA1c increase was 0.23%. No significant changes were observed in BMI or body composition. Six patients had dysglucosis, 4 (11.7%) developed diabetes and 2 (5.7%) impaired fasting glucose. However, no predefined baseline traits were significantly related to the metabolic derangements, including glucocorticoid replacement.
In conclusion, our results indicate that long-term GHR mildly increases FG and HbA1c. Current hydrocortisone replacement regime was not associated with dysglucosis in our series.

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P713

Clinical case of silent corticotropinoma

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Silent corticotropinomas (SCAs) are a subtype of non-functioning pituitary adenomas (NFPAs) demonstrating positive immunoreactivity for ACTH, but without clinical or biochemical features of hypercortisolism. SCAs typically present as macroadenomas with symptoms related to a local mass effect. In some cases SCAs can transform into hormonal active formations with gradually developing symptoms of hypercortisolism.
The aim
To describe a patient with NFPA who over time developed a biochemical only presence of hypercortisolism.
A case description
A young woman presented at the age of 25 years with headaches and oligomenorrhea. The hormonal tests showed the moderate hyperprolactinemia 28.5 ng/ml ($n < 19.5$) with the absence of macroprolactin, normal levels of TSH, IGF1, ACTH, cortisol, LH, FSH, the MRI revealed pituitary macroadenoma 1×1.2 cm with the trend toward suprasellar extension. Short-term medical treatment with low doses of dopamine agonists resulted in drop of prolactin levels below reference values and normalization of menstrual function, which was consistent with the diagnosis of NFPA with hyperprolactinemia due to local mass effect. At the next follow-up 2 years later the patient complained of headaches, weakness and psycho-emotional instability. Lab data revealed high levels of ACTH/cortisol, absence of cortisol suppression at the 1 mg dexamethasone test, and a significant suppression at 8 mg dexamethasone test. The MRI-scan didn't detect any growth of the adenoma. Remarkably, the patient did not have any clinical features of hypercortisolism (BMI 20.5 kg/m^2, normal fat distribution, absence of striae and hyperpigmentation, normal levels of blood pressure and blood sugar levels, osteopenia, but no osteoporosis or fractures). At age of 28 years the patient underwent a transnasal adenomectomy with developing of adrenal insufficiency on the second day after operation. The immunohistochemical analysis showed ACTH-immunopositivity which confirmed corticotropinoma, low expression of GH and was negative for prolactin and CRH.
Conclusions
This case demonstrates the gradual development of subclinical Cushing's disease from NFPA during a period of 3 years. The current diagnosis NFPAs should be made with a high degree of caution and repeated assessment of potential hormonal activity of adenoma to ensure timely treatment.

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P714

Central diabetes insipidus, as the first sign of Langerhans cell histiocytosis in an adult

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Introduction
Langerhans cell histiocytosis (LCH) is a rare granulomatous disease of unknown etiology. LCH may be localized or be a systemic disease. The diagnosis is frecuent in pediatric age. In adults, infiltration is most frecuently in bones, lungs and skin, and shows particular predilection for hypothalamo-pituitary axis.
Case
A 51 years old man was referred due to polyuria of ~15 l of daily urine output. His past medical history revealed bronchiectasis and skin problems. Laboratory test demonstrated hypernatremia, decreased urinary and increased serum osmolarities. Water deprivation test was not held due to severe signs of dehydration. Pituitary MRI showed enhancing pituitary signal with thickened stalk and loss of the bright spot of the neurohypophysis. Desmopressin was started with a presumptive diagnosis of insipidus diabetes and urine output and biochemical tests returned to normal. Anterior pituitary hormones were normal. Months later, he presents temporal cephalea and MRI revealed two subcortical lesions. Neurosurgery was practiced and histology confirmed Langerhans cell proliferation. His skin and lungs were also affected. Levothyroxine and testosterone therapy were commenced due to progressive failure of the gonadal and the thyroid axis. Nowadays, he has begun chemotherapy and corticotherapy.
Conclusions
i) Although LCH is mainly considered a pediatric disease, it may be diagnosed in adults, ii) the difficulty in making an accurate diagnosis is reflected in the long time that takes from the onset of the symtoms to the diagnosis, due to the fact that patients visit many different specialistes, the lack of clinical suspicion, and the variable characteristics of the disease, and iii) LCH is a multisystemic disease and multidisciplinary management is important.

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P715

RET codon 618 mutation is the most frequent genotype in saudi families with multiple endocrine neoplasia type 2a and familial medullary thyroid carcinoma

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Background and objectives
Certain diseases, such as multiple endocrine neoplasia type 2A (MEN2A), MEN2B, familial and sporadic medullary thyroid carcinoma (MTC) and renal dysgenesis are related to abnormalities of the RET protein. Our aim was to evaluate the frequency of *RET* mutation in ten Saudi families with MEN2A and familial medullary thyroid carcinoma.
Design and setting
Cross-sectional prospective study of patients followed up at King Abdul-Aziz University Hospital and King Abdul-Aziz Medical City, Jeddah between March 2001 and March 2011.
Patients and methods
Genomic DNA was isolated from peripheral blood leucocytes of all the subjects using standard procedures. Exons 10, 11, 13, 14 and 16 of the *RET* proto-oncogene were analyzed by single-strand conformation polymorphism, direct DNA sequencing and/or restriction enzyme analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences.
Results
Seventy-nine subjects were screened for *RET* mutation. Forty-three of the subjects with hereditary MTC were enrolled in this study. MEN2A was identified in 25 cases; MTC was diagnosed in all 25 cases (100%), pheochromocytoma in 13 cases (52%) and hyperparathyroidism in 4 cases (16%). The most frequent genotype in patients with MEN2A syndrome was codon 618 mutation (46.6%), followed by 634 mutation (44.2%). Among the five families with MEN2A, three had a mutation at codon 634, while two had a mutation at codon 618.
Conclusion
The most frequent *RET* proto-oncogene mutation in our series was in codon 618 (exon 10).

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P716

Neurological manifestations of Vitamin D deficiency, is there any significant clinical correlation?

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Objective
The objective of this study was to investigate the correlation between the neurological manifestations of Vitamin D deficiency and the levels of 25(OH) D and bone profile.
Methods
We conducted a case series study in patients with osteomalacia who were followed up at King Abdulaziz Medical City, Jeddah, between January 2010 and December 2011. We collected information on demographic data, etiological factors for Vitamin D deficiency, clinical presentations (typical and neurological), and radiological findings. T test was used to determine whether there was a correlation between the neurological manifestations of Vitamin D deficiency and vitamin D levels and bone profile. A P-value < 0.05 was considered significant.
Results
Sixty patients were enrolled in the study. Atypical presentations included progressive muscle weakness (proximal more than distal) in 73% of the cases and gait disturbances in 61.7% of the patients. There was no significant correlation between neurological manifestations and the bone profile or vitamin D levels. Significant correlations existed only between the inability to walk and the levels of serum calcium and phosphate, with P-values of 0.043 and 0.037, respectively.

Conclusions
Neurological manifestations of Vitamin D deficiency are not correlated with the levels of 25(OH) D or bone profile.
Keywords
Vitamin D deficiency, vitamin D, proximal myopathy, bone profile.
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P717

Craniopharyngioma – a diagnosis not to be missed

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Introduction
Craniopharyngiomas are intracranial tumors that develop from Rathke's pouch rests of epithelium. They have a bimodal age distribution, with peak incidence at childhood and in the adult/elderly, although in our series we have more patients in a middle peak. Usually they are benign but responsible for significant morbidity, particularly when located near critical structures such as optic chiasm, pituitary gland and hypothalamus, and thus might cause visual, neurological and endocrine deficits.
Case report
A 24-year-old male, asymptomatic until January/2011, when started complaining of progressive visual impairment of the right eye without other neuro-ophthalmological changes neither hormonal dysfunction symptoms. In July/2011, he reported amaurosis of the right eye. The hormonal evaluation revealed only subclinical hypothyroidism, and he was started on levothyroxine therapy (50 μg/day). Pituitary MRI showed a sellar and supra-sellar cystic lesion with carotid involvement. This lesion was not completely excised, because of its adherence to internal carotid. Histology revealed craniopharyngioma adamantinomatous type. The patient was discharged with diagnosis of hypothyroidism and central diabetes insipidus, on levothyroxine supplementation and desmopressin therapy (0.2 mg/day). The post-operative endocrinological revaluation showed panhypopituitarism, requiring adjustment of levothyroxine besides testosterone and prednisone supplementation. The pituitary follow-up MRI showed enlargement of the suprasellar cistern and sella turcica, probably related to sequelae of surgery, tumor-adjacent residues and optic chiasm with scoop bottom and with discreet focus of the contrast on the inferior right location. Clinically the patient maintains visual impairment of the right eye ($<1/10$), relative afferent pupillary defect, and pallor of optic disc in ophthalmoscopy, without other neurological or endocrine symptoms.
Conclusion
Craniopharyngiomas are rare tumors, often with suprasellar extension. Early diagnosis and treatment require a high diagnostic accuracy when dealing with visual impairment, neurological and hormonal symptoms. They tend to invade locally and relapse after treatment, requiring a long follow-up.
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P718

Features of family inheritance in patients of non-functional pituitary adenomas in Uzbekistan

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Aim
To study features of family inheritance in patients of non-functional pituitary adenomas (NFPA) in Uzbekistan.
Materials and methods
We included 71 patients with NFPA, intrasellar localization (mean size >3 mm). Mean age was from 18 to 70 years old (range 44.5 ± 3.85 years). Of 71 patients, 30 (42.3%) were men and 41 (57.7%) were women.
All patients underwent clinical and biochemical evaluations including endocrine check, lipid profile, hormonal profile (LH, FSH, prolactin, STH, TSH, etc.), genitalia ultrasonography, CT/MRI of pituitary, visual fields, genetic methods if investigation (FNO-&, proapoptosis protein of gene p53, antiapoptosis protein of gene of bcl-2, activity of factors of angiogenesis-VGFE, VGFER-2, eNOS-3) and study of family anamnesis.
Results
In this investigation, we disjointed 71 patients with NFPA into two groups: group 1 – without hereditary load – 50 (70.4%) and group 2 with hereditary load – 21 (29.6%), including panmixia – 9 (2.7%) and inbreeding –12 (16.9%) patients.
The clinical analysis submitted that in patients with inbreeding, the onset of disease has been diagnosed at 3–5 years earlier, more often in young age – before 28–35 years, than in patients with panmixia.
Conclusion
i) On the base of in-depth study of features of NFPA, we found in our patients with NFPA 2 types of family inheritance: panmixia (73.8%) and inbreeding (26.8%).
ii) In patients with inbreeding more frequently happen in women with mean size of tumor in the range 8–10 mm, but in patients with panmixia – equality in men and women with mean size until 8 mm.
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P718.1

Abstract withdrawn.

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Nuclear receptors and signal transduction

P719

The nuclear corepressor NCoR is an essential mediator of the anti-tumorigenic and anti-metastatic actions of the thyroid hormone receptor

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Reduced expression or activity of the thyroid hormone receptors (TRs) are common events in cancer, suggesting that these receptors could act as tumor suppressors. We have previously shown that TRβ expression in SK-hep1 hepatocarcinoma cells (SK-TRβ cells), reduces tumor growth and strikingly inhibits invasion, extravasation, and metastasis formation in nude mice. These effects could be related to a decreased expression in these cells of prometastatic genes, such as COX2, MMP9 or ID1. We have now found that transcripts for these genes are also decreased in tumors and metastasis originated by SK-TRβ cells in nude mice. Using transient transfection assays, we have localized CRE, AP1 and SP1 sites in their promoters that appear to mediate the inhibitory effect of TRβ. This suggests that cross-talk with other transcription factors plays an important role in the repression of prometastatic gene transcription by the receptor. One mechanism that could explain suppression of prometastatic gene expression by TRβ is the recruitment of corepressor complexes. Indeed, we found that expression of the corepressor NcoR is significantly higher in SK-TRβ cells than in parental cells and is also increased in tumors and metastasis. NcoR silencing with interference RNA produces a strong increase in transcript levels of several prometastatic genes and enhances cellular invasion in matrigel assays. These effects appear to be specific for NCoR, since depletion of the corepressor SMRT is ineffective. Furthermore, NCoR silencing reverses significantly the effect of TRβ expression, enhancing tumor growth and invasion, extravasation and metastasis development when cells are inoculated into nude mice. These changes are associated with increased expression of prometastatic genes in the tumors and in the metastatic injuries.
These results demonstrate the essential role of the corepressor NcoR in the tumor suppressive actions of TRβ, and suggest the importance of the corepressor as a potential therapeutic target in cancer.
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P720

Coordination of 17β-estradiol-dependent cell proliferation requires diverse post-translational modifications

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The sex hormone 17β-estradiol (E2) exerts its pleiotropic effects through the binding to the ligand-activated transcription factor estrogen receptor alpha (ERa). The E2:ERa complex regulates several physiological processes including cell survival and proliferation through transcriptional (i.e., estrogen responsive element (ERE)-based gene transcription) and non-transcriptional membrane-initiated effects (i.e., activation of signalling cascades).
Many post-translational modifications occur on ERa and are regulated by E2. Indeed, E2 induces ERa phosphorylation that facilitates ERa-dependent gene transcription while the hormone reduces ERa palmitoylation, thus modulating the amount of the receptor located at the plasma membrane and the E2 signalling to cell proliferation. The ERa is also an ubiquitinated protein: ERa polyubiquitination (polyUbq) increases upon E2 binding and E2-dependent ERa degradation occurs in parallel to the appearance of the E2-evoked physiological effects.
However, the role of ERa post-translational modifications in the regulation of the E2-dependent cell proliferation is poorly appreciated. Therefore, we analyzed here how ERa phosphorylation, palmitoylation and ubiquitination influence E2-induced cell proliferation in an integrated manner.
Our results demonstrate that the polyUbq-based ERa degradation cross-talks with receptor phosphorylation and palmitoylation and is required for the E2-dependent control of cell proliferation. Furthermore, the lack of ERa palmitoylation fastens E2-induced polyUbq-dependent ERa degradation and prevents both receptor phosphorylation and E2-dependent cell proliferation.
Therefore, these data demonstrate that a code of diverse post-translational modifications occurs on ERa and uncover a new model of E2: ERa cellular signalling in which the E2-dependent control of ERa post-translational modifications finely coordinates the E2 ability to regulate cell proliferation.

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P721

Non-proteolytic ubiquitin-based signalling regulates estrogen receptor α activities

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Protein ubiquitination modulates many physiological processes (e.g. cell proliferation). The ubiquitin-based signalling consists in both proteolytic and non-proteolytic functions: the first ones require modification of the target protein with polyubiquitination, a modification that induces the activation of the 26S-proteasome, whereas the second ones can be based on protein monoubiquitination. The recognition of ubiquitination diversity is dependent on specific Ub receptors that bind to the ubiquitinated protein by contacting the Ub-modification through specific ubiquitin-binding domains (UBDs).
Estrogen receptor α (ERα) is a ligand-activated nuclear receptor that mediates the cellular effects of the steroid hormone 17β-estradiol (E_2). The ERα-based signalling is a function of receptor intracellular localization. While ERα nuclear localization is required for E_2-induced gene transcription, the ERα extra-nuclear localization is necessary to trigger the rapid activation of several signalling kinase cascades (e.g. ERK/MAPK, PI3K/AKT).
Recent data provided the initial evidence that proteolytic and non-proteolytic Ub-based functions modulate ERα activities. ERα is a monoubiquitinated protein but the monoubiquitination-dependent regulation of the ERα activities that lead to the activation of cell proliferation are not known. Furthermore, the fact that ERα is a monoubiquitinated protein opens the possibility that an UBD could be present within the ERα structure and that the receptor could behave as an ubiquitin receptor. However, whether non-covalent Ub: ERα binding could occur and play a role in E_2: ERα signalling is unknown.
Here, we show that mutation of the ERα monoubiquitination sites prevents the E_2: ERα-mediated activation of signalling pathways to cell proliferation. In addition, a previously unrecognized Ub-binding surface has been found within the ERα and contribute to the E_2 transcriptional activity.
Altogether, these data indicate that the ERα belongs to the Ub-based signalling network and that receptor monoubiquitination as well as non-covalent Ub binding regulate ERα functions in a non-proteolytic manner.

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P722

The thyroid hormone receptors inhibit invasive and fibrotic responses to TGFβ by transcriptional cross-talk with smad-transcription factors

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Transforming growth factor β (TGFβ) signals through activation of Smad transcription factors, which bind to Smad binding elements (SBEs) in target genes. TGFβ can promote cancer cell proliferation and invasion, and plays a key role in the pathogenesis of scleroderma and other fibrotic disorders. We have previously shown that the thyroid hormone receptors (TRs) can block tumor growth and invasion. We now have analyzed the possibility that TRs could antagonize TGFβ-ependent responses. We found that liganded TRs block transactivation of SBE-containing reporter plasmids by TGFβ, and repress transcription of endogenous TGFβ target genes. The thyroid hormone T_3 reduces Smad phosphorylation by TGFβ and causes a limited inhibition of Smads translocation to the nucleus. There is a direct and constitutive protein-protein interaction between TR and Smad transcription factors that could be involved in the observed effects. In chromatin immunoprecipitation assays (ChIP) with SBE-containing promoters, T_3 inhibits TGFβ-dependent recruitment of Smads, reduces acetylated histones and induces recruitment of histone decetylase 3 (HDAC3). The hormone is also able to block TGFβ-dependent proliferation and migration of cultured cancer cells. The potential anti-fibrotic effect of the TRs was investigated in mice using a model of skin fibrosis induced by subcutaneous injections of bleomycin. In euthyroid mice bleomycin caused a significant increase in dermal thickness, hair loss and high packaging of collagen fibers, characteristic of areas of dermal scleroderma and fibrosis. Topical application of T_3 significantly attenuated fibrosis, with a decrease of dermis thickness, reduced compactation of bundle collagen fibers and more extracellular matrix, associated to a low collagenization of subcutaneous cellular tissue. These results demonstrate that T_3 blocks transcriptional responses to TGFβ, and suggest that some of the actions of the thyroid hormone receptors, both *in vivo* and in cultured cells, can involve repression of the activity of the TGFβ signalling pathway.

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P723

5/6 Nephrectomy reduces muscle mitochondria in mice as to decrease exercise endurance associated with further exacerbation by dietary protein

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Introduction
Chronic kidney disease impairs physical performance from an early stage of disease, and the impairment is a critical medical problem because lowered physical activity correlates with renal prognosis and mortality, and constitutes an independent cardiovascular risk for chronic kidney disease patients, with as high an impact as hypertension and diabetes. In this study, the time course and mechanism of muscle insufficiency in renal failure were investigated using 5/6 nephrectomized (5/6Nx) mice, a model of mild chronic kidney disease. Furthermore, the effect of high-protein diet on chronic kidney disease was examined.
Methods
C57bl/6 mice which had undergone 5/6 nephrectomy at 6–7 weeks were examined for physical performance in young (16–20 weeks old) and aged (48–52 weeks old) groups. Protein adjusted diets were fed from 8 weeks old.
Results
A decrease in muscle mitochondria and running distance was identified in young 5/6Nx mice, despite the preservation of muscle volume and power. Thereafter, a decrease in muscle volume associated with a reduction in muscle power became apparent in aged 5/6Nx mice. A high-protein diet feeding from 8 weeks old increased muscle volume and power in the mice; however, it further decreased running distance, associated with a decrease in the activity of pyruvate dehydrogenase which promotes aerobic glycolysis. Activation of pyruvate dehydrogenase by dichroloacetate effectively recovered running distance which was decreased by dietary protein.
Conclusion
A decrease in muscle mitochondria was identified as a new mechanism for the reduction in exercise endurance of 5/6 nephrectomy mice. Decreased pyruvate

dehydrogenase activity accounted for the further reduction in exercise endurance by dietary protein. These findings clarify the mechanism of muscle insufficiency in renal failure and suggest activation of muscle mitochondria as a potential strategy for the improvement of physical performance of patients with chronic kidney disease.

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P724

Effects of *Tribulus terrestris* on immune function in over-trained rats and its mechanism: the role of glucocorticoid and glucocorticoid receptor

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Purpose
To investigate the effects of *Tribulus terrestris* (TT) on immune function in over-trained rats and explore the mechanisms – the role of glucocorticoid (GC) and glucocorticoid receptor (GR).
Methods
Thirty male SD rats were randomly divided into control group, over-trained group and over-trained + TT group. The last two groups were trained on a motor-driven treadmill with a progressively increased load for 6 weeks. The over-trained + TT rats took the medicine by gastric irrigation while the others ingested the same amount of saline. The levels of plasma testosterone and corticosterone were measured by ELISA. The numbers of CD_3^+, CD_4^+ and CD_8^+ T cells and the levels of IFNγ and IL4 were detected by flow cytometry.
Results
Distinct decreases of body weight, testosterone:corticosterone (T:C) ratio, numbers of CD_3^+T cells and natural killing (NK) cells were found in the over-trained rats compared with that of non-exercise rats, indicating successful establishement of exercise-related immunosuppression; supplement of TT lead to significant increases of T:C ratio, CD_4^+:CD_8^+ ratio and the amounts of CD_8^+ cells, NK cells, NKT cells in the over-trained rats; down-regulation of IFNγ:IL4 ratio was shown in the over-trained rats, which meant Th1/Th2 imbalance (shift to Th2), and supplement of TT increased IFNγ:IL4 ratio; the level of plasma GC in the over-trained + TT rats was decreased obviously (about 50% of over-trained rats); the protein levels of GR in liver decreased obviously in the over-trained rats while TT increased the expression of GR in liver.
Conclusion
These results indicated that: supplement of TT improved the suppressed immune function resulted from over-training in rats, including increases of CD_4^+:CD_8^+ ratio and the amounts of NK, NKT cells and retrieval of Th1/Th2 balance; the improved effect of TT on suppressed immune function in over-trained rats might be related to the increase of GR and decrease of GC.

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P725

Nuclear receptor 'master' coactivators of physiology and pathology

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Nuclear receptors control gene expression by recruiting transcriptional coactivators (or corepressors). The coactivators are 'master regulators' that coordinately activate multiple distinct transcription factors and target genes and pathways to control major physiologic processes such as reproduction, inflammation, metabolism and growth. Because of their central role as 'nodes' of regulation, coactivators are major targets in the development of numerous inherited and acquired endocrine-related pathologies such as infertility, endometriosis, disorders of carbohydrate, lipid and protein metabolism, and numerous cancers. Metabolism and growth are especially prominent pathways for coordinate regulation by coactivators such as SRC-2 and SRC-3. The pleiotropic functions of coactivators in pathways are the result of combinatorial posttranslational modifications of the proteins via enzyme cascades, in conjunction with certain biological isoforms of the proteins. In metabolic diseases and cancers, the intracellular concentrations and the PTM-directed 'activities' of the coactivator proteins are critical for 'driving' the transcription-dependent physiological outcomes, However, in the case of the cancer cell's motility or in endometriosis, it is the coactivator protein's isoforms that are the major mediators of the disease progression. Thus, as a class, the coactivator proteins provide important insights to polygenic diseases. They also may represent new 'first-in-class' types of potential targets for therapeutic intervention.

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Obesity

P726

The lack of Nur77 affects energy and glucose metabolism in females fed on high fat diet

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Nur77 is an orphan nuclear receptor included in the NR4A family. This protein has been studied as a transcriptional regulator of glucose metabolism in different tissues. We aimed to investigate the endogenous role of Nur77 on energy and metabolic homeostasis. For this purpose, we used male and female Nur77-deficient mice and their wild-type littermates. We challenged them to standard diet and high fat diet, and analyzed different parameters involved in energy balance (food intake, energy expenditure, locomotor activity and respiratory quotient) and glucose metabolism (glucose and insulin tolerance tests).
We found that the lack of Nur77 causes a dimorphic response in the metabolic phenotype of mice fed with high fat diet, being the females more sensitive to high fat diet, while male mice lacking Nur77 were similar to their controls. Female Nur77 deficient mice gained more fat mass, an effect that may be explained by the lower energy expenditure in comparison to female wild types. Consistent with the higher amount of fat mass, female Nur77 deficient mice showed a lower insulin sensitivity, with no changes in glucose tolerance.
In conclusion, our findings indicate that Nur77 is a potentially important physiological mediator of body weight and insulin sensitivity, particularly in female mice.

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P727

Abstract unavailable.

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P728

Serum- and glucocorticoid-regulated kinase 1 in obesity-related adipose tissue and peripheral inflammation

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The serum- and glucocorticoid-regulated kinase 1 (Sgk1) is a direct transcriptional target of glucocorticoids and is post-translationally activated by the insulin/IGF1 pathway. Distinct polymorphisms in the Sgk1 gene are

associated with increased body weight and type 2 diabetes. Here we investigate the expression and regulation of Sgk1 in human obesity.
In both omental and subcutaneous human adipose tissue, Sgk1 expression is significantly increased in 20 obese patients compared to 20 age- and gender-matched controls. Sgk1 protein expression is mainly localised to adipose tissue macrophages. The expression of Sgk1 mRNA in subcutaneous adipose tissue correlates with waist circumference, weight, fat mass and HOMA insulin resistance index, as well as with circulating levels of C-reactive protein (CRP), leptin, interleukin 6 (IL6) and macrophage inflammatory protein 1α (MIP1α). Multiple regression analysis shows that waist circumference, weight, CRP and MIP1α independently predict Sgk1 expression in adipose tissue.
In peripheral blood mononuclear cells of eight obese patients compared to eight age- and gender-matched controls, Sgk1 expression is significantly higher in obese subjects. Monocyte-to-macrophage differentiation by phorbol 12-myristate 13-acetate (PMA) strongly induces Sgk1 mRNA expression in the THP1 monocytic cell line, parallel to increasing IL6 transcription. Lipopolysaccharide (LPS) significantly up-regulates Sgk1 mRNA expression in THP1 monocytes, but has no additional effect on PMA-stimulated Sgk1 in THP1 cells.
Taken together, we show increased Sgk1 expression in adipose tissue macrophages as well as circulating mononuclear cells of obese subjects compared to non-obese controls, presenting the first association between Sgk1 and obesity-related inflammation. In addition, our data identify PMA and LPS as novel regulators of Sgk1 in monocytic cells and suggest a role of Sgk1 in monocyte-to-macrophage differentiation. Further investigation of Sgk1 function in this context might be of therapeutical relevance in obesity-related inflammation and its comorbidities.

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P729

New insights on molecular mechanisms regulating hepatic sex hormone-binding globulin production: clinical implications in obesity and type 2 diabetes

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Human sex hormone-binding globulin (SHBG) is produced and secreted by the liver and it binds androgens and estrogens with high affinity. In blood, SHBG acts as a carrier of these sex steroids and regulates their bioavailability. Low plasma SHBG levels are associated with obesity, abdominal adiposity and metabolic syndrome, and predict the development of type 2 diabetes. In addition, an inverse relationship between plasma SHBG levels and risk of cardiovascular disease has been reported.
The SHBG gene has changed its tissue expression and therefore its function during the evolution. Rodents express the SHBG gene in the Sertoli cells of the testis. While in humans, the SHBG gene is expressed in the liver and in the germ cells of the testis. This change of function and tissue expression can be explained by the appearance during evolution of new footprinted regions in the human promoter and an alternative promoter. The generation of the human SHBG transgenic mice has allowed us to study the SHBG expression and regulation *in vivo*. We have used these mice and HepG2 cells to provide evidence that SHBG expression is downregulated by monosaccharides describing the underlying molecular mechanism. We have also demonstrated that proinflammatory cytokines (TNFα and IL1β) downregulates SHBG production by reducing HNF4α levels. These findings give a new explanation by which those patients suffering from chronic inflammation diseases such as obesity and type 2 diabetes have also low levels of SHBG. Noteworthy, our *in vitro* and *in vivo* studies showed that insulin does not regulate hepatic SHBG production. This finding contravenes the classic assumption that insulin is the primary regulator of SHBG production in the liver.
Finally, we have generated a new mouse model by crossing the human SHBG transgenic mice with the db/db mice. The generation of these mice has allowed us to study the regulation of the human SHBG during the development of obesity. As occurs in human obese subjects, these mice showed low plasma SHBG levels as well as low total and free testosterone levels. This model will permit us to further explore SHBG regulation and to design new therapeutic approaches.

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P730

Obesity: a paradox in the mortality of the elderly

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Introduction
The effect of BMI on mortality amongst the elderly may be different from younger adults. Thus, this study aims to determine the relationship between BMI and its 5-year changes on all-cause and cause-specific mortality in the elderly.
Methods
During 1992–1994, the Blue Mountains Eye Study recruited 3654 residents aged ≥49 years in Sydney, Australia. Of these, 75% of survivors returned for follow-up at 5 years. We initially examined the relationship between baseline BMI and mortality nonparametrically using cubic spline. Cox and competing risk models were used to assess associations of baseline BMI and its 5-year changes with all-cause and cause-specific mortality.
Results
Underweight persons were more likely to be older (mean 66.9, s.d. 9.9 years) and predominantly female (74%). Conversely, obese persons were more likely to be younger (mean 63.7, s.d. 8.3 years), and to have a history of pre-existing disease (hypertension, diabetes, angina, AMI and stroke; 54.5%). Amongst subjects without pre-existing disease, the relationship between baseline BMI and all-cause mortality was U-shaped, with the underweight and obese groups being predisposed to have a greater risk of death. In particular, obesity was associated with coronary heart disease (CHD; hazard ratio (HR) 2.78, 95% CI: 1.34–5.77) and cancer (HR 1.90, 95% CI: 1.08–3.12) deaths. For subjects with pre-existing disease, however, an inverse relationship was observed, with the underweight having a lower risk of death. Five-year reductions in BMI were associated with all-cause, cancer and CHD deaths.
Conclusion
Obesity affects all-cause, CHD and cancer mortality only amongst the elderly without pre-existing disease. BMI loss was associated with major causes of mortality. Understanding the varying impact of obesity on mortality amongst the elderly with and without pre-existing disease will provide clinicians and public health policymakers with critical evidence for disease management, resource planning and allocation.

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P731

Brite adipocytes: a role for cannabinoid receptor type 1 in brown adipose cell recruitment

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Recent data showed the existence of a specific adipocyte population composed of 'brite' cells in white adipose tissue (WAT), characterized by a peculiar gene expression pattern including PRDM16. Despite showing a low basal UCP1 expression, these cells display higher UCP1 expression when stimulated. In view of the involvement of the endocannabinoid system in energy homeostasis, the aim of our work is to establish whether the CB1 receptor could be a target for brown phenotype induction *in vivo*.
We analyzed UCP1 and PRDM16 mRNA expression by QT-RTPCR in brown adipose tissue (BAT), white subcutaneous inguinal (scWAT) and visceral epididymal (eWAT) adipose tissues, obtained from CB1KO (CBN) and Rimonabant-treated (10 mg/kg per day i.p., 4 weeks) mice vs respective controls (CT), undergoing standard diet (SD) or high-fat diet (HFD) from the 8th to 20th week of age. In CT mice, HFD significantly stimulates UCP1 mRNA in all adipose depots (eWAT $P<0.001$; scWAT and BAT $P<0.01$) when compared to SD mice, whereas PRDM16 expression is increased only in eWAT depot ($P<0.05$), suggesting a tissue-specific gene expression response induced by HFD. We observed constant and similar gene expression modulation in CBN and Rimonabant treated animals. Comparing CT and CBN/Rimonabant mice, relevant changes in gene expression were found in scWAT where CB1 signaling inhibition induced a strong increase in UCP1 expression ($P<0.01$) regardless of diet type, whereas CB1 blockade induced PRDM16 increase ($P<0.05$) only in HFD condition. These data, together with the finding of the sole increase of CB1 expression in HFD vs SD in scWAT ($P<0.01$), strengthen the hypothesis that CB1 receptor antagonization may modulate brown-cell recruitment mechanisms on specific WAT depots. Further studies are underway to complete the characterization of the gene expression pattern of these putative 'brite' cells.

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P732

Metabolic side effects of second generation antipsychotic drugs in adolescents

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Introduction
Although for the last 2 decades we have witnessed a rise in the use of second generation antipsychotic treatment in children and adolescents suffering from psychiatric disorders we do not have enough studies in specialty literature to estimate the risks associated with this medication. Recent data and also our clinical experience shows that second generation antipsychotic drugs administration correlates with considerable weight gain and also with altering of glucide, lipide and other metabolic markers. The utility of antipsychotic treatment in psychotic disorders and schizophrenia is well known. But does it outweigh the risks? Studies that evaluate the risk/benefit ratio of antipsychotic treatment in early-onset and very-early-onset schizophrenia are much needed.
Design/methods
In the study there were included 19 adolescents aged 12–17 years old who were diagnosed with early-onset and very-early-onset schizophrenia according to DSM-IV criteria and KIDDIE-SADS scales. These patients were treated with risperidone 2–4 mg/day for schizophrenia and trihexiphenidinum (2–4 mg/day) for extrapiramidal sindrome prophilaxy. The following metabolic parameters were followed at baseline, 8 weeks and 6 months: fasting glucose, insuline, cholesterole: HDL, LDL, and plasma trigliceige concentration, hepatic markers: ALT and AST as well as TSH and weight.
Results
At 8 weeks there were no consistent modifications in weight or metabolic parameters but at the 6 months measurement 58% of the patients had gained more than 5kg and there could be observed slight elevations in plasma trigliceride concentration and cholesterole levels.
Conclusions
The small number of patients is for clear a limitation, therefore we find that studies on bigger populations are necessary. Nevertheless this results show the need for an multidisciplinary – psychiatrist, endocrinologist, family doctor – approach of patients undergoing antipsychotic treatment and especially during a period marked by growth and change like adolescence.

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P733

Gastric banding vs gastric bypass: evolution of anthropometric parameters and related comorbidities during the 2 years after surgery

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Introduction
Bariatric surgery has proved to be effective in the management of morbid obesity. Our purpose was to compare the results obtained with adjustable gastric banding (GB) and Roux-en-Y Gastric Bypass (RYGB) during 2 years of follow-up after surgery, with regard to anthropometric parameters and related comorbidities.
Methods
We evaluated prospectively patients who underwent GB and RYGB between 2004 and 2010 and compared the evolution of anthropometric parameters, prevalence of type 2 diabetes mellitus (DM), hyperlipidemia and hypertension during 2 years of follow-up.
Results
109 patients were submitted to bariatric surgery: 51 patients to GB (mean age 48.4 ± 10.6, 84.3% women) and 58 to RYGB (mean age 45.2 ± 9.2, 86.2% women). There were no statistically significant differences between the two groups regarding age, gender, weight, BMI, weight excess, BMI excess and prevalence of DM and hypertension before surgery; hyperlipidemia was more frequent in the RYGB group (46.6 vs 27.5%). Two years after surgery, percentages of excess weight and BMI losses were higher with RYGB (70.9 ± 14.0 vs 38.5 ± 22.3% and 83.7 ± 7.8 vs 45.6 ± 26.3% respectively). In the GB group, DM remitted in 75% of the patients, hypertension in 61.1% and hyperlipidemia in 64.3%; in the RYGB group, the respective percentages were 65, 61.1 and 85.2%.
Patients who didn't achieve resolution of their comorbidities improved and needed less medication. The reduction of the prevalence of DM, hypertension and hyperlipidemia was significant in both groups and the post-surgical prevalence of those comorbidities was not significantly different between GB and RYGB.
Conclusions
Our study shows that RYGB results in higher weight and BMI loss than GB until the second year after surgery. Both procedures allow a significant resolution of obesity-related comorbidities.

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P734

Obesity-induced hepatic and placental inflammation are absent in obese gestating mice compared to control fed dams

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Introduction
Maternal obesity is associated with increased risk of metabolic dysfunction in the offspring. It is not clear which physiological aspects of the obese state that cause this metabolic programming. Obesity causes many metabolic changes but also low grade inflammation. In this study, we have determined if increased low grade inflammation was present in obese dams compared to controls dams during gestation.
Methods
Female C57BL/6 mice were fed either a standard chow diet (3% fat) or a highly palatable obesogenic diet consisting of a high fat pellet diet (20% fat) supplemented with sweetened condensed milk. After 6 weeks on the diets, half the mice ($n=12$) were sacrificed and the remaining half were mated and sacrificed on gestation day 18 ($n=8$). Blood and tissues were collected for analysis.
Results
The obesogenic diet increased adiposity, adipocyte size and leptin levels both in the pre-gestating and gestating state. There was also a tendency for increased

hepatic lipid accumulation in obese mice. Body weight was increased in pre-gestating obese mice, but at the end of gestation there was no change in body weight between control and obese dams. Insulin levels were higher in pre-gestating obese dams. During gestation, a marked increase in the control dams, not seen in the obese, equalized this difference. Blood glucose levels were unaffected by diet or gestation. Local inflammation was assayed by macrophage count in liver and placenta. Hepatic macrophage count was in general reduced by gestation but only obese mice showed a significantly lower macrophage count during gestation, due to an elevated count prior to gestation. Placenta macrophage count was unaffected by the diet.

Conclusion

Obese dams were found not to express increased inflammation in placenta and liver compared to lean dams, despite profound hepatic inflammation before gestation. Thus, the diet-induced inflammation is not maintained during gestation.

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P735

Serum IL18 concentration is associated with resting energy expenditure independently of BMI

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Introduction

Numerous studies indicate an association between low-grade chronic inflammation and predisposition to type 2 diabetes and atherosclerosis. IL18 is a proinflammatory cytokine with proatherogenic properties. Existing evidence indicated that circulating IL18 was associated with insulin resistance, metabolic syndrome and type 2 diabetes. Some data suggest that endogenous IL18 signaling modulates food intake, metabolism and adiposity, as homeostatic regulator. The aim of the present study was to estimate the relationship between serum IL18 concentration and resting energy expenditure (REE).

Methods

Our study involved 60 young (age: 23.43 ± 2.62 years), apparently healthy men with normal glucose tolerance. Anthropometric measurements, blood biochemical analysis, euglycemic hyperinsulinemic clamp and indirect calorimetry before and during the clamp were performed in the studied group.

Results

The serum concentrations of IL18 was 259.95 ± 98.36 pg/ml. We found that serum IL18 positively correlated with BMI ($r=0.26$, $P=0.04$), fasting serum insulin ($r=0.26$, $P=0.047$) and free fatty acids after the clamp ($r=0.50$, $P=0.001$). A significant association between basal and post-clamp REE with serum IL18 ($r=0.40$, $P=0.001$ and $r=0.26$, $P=0.043$, respectively) was observed in the studied group. In multiple regression analysis the relationship between IL18 and REE was independent of BMI ($\beta=0.22$, $P=0.008$).

Conclusion

Our data indicate that serum IL18 concentration is associated with resting energy expenditure independently of BMI.

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P736

High fat diet induces site specific resistance to LPS-stimulated STAT3 activation in the CNS

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Hypophagia and increased energy expenditure under inflammatory condition, such as observed after bacterial lipopolysaccharide (LPS) administration, are associated with activation of JAK2–STAT3 signaling in the CNS. High fat diet (HFD) is known to induce hypothalamic resistance to leptin signaling mediated by JAK2–STAT3 pathway. In this study we investigated the expression of p-STAT3 in the hypothalamus and brainstem of HFD treated animals under LPS (100 μg/kg) stimulation. Wistar rats fed standard/low-fat diet (3.95 kcal/g) or HFD (6.3 kcal/g) for 8 weeks were assigned into control diet-saline, control diet-LPS, HFD-saline and HFD-LPS groups. LPS reduced feeding in the control diet, but not in the HFD group. In control diet fed rats, LPS increased the STAT3 phosphorylation in the arcuate nucleus and ventromedial hypothalamic nucleus, but not in the HFD group. HFD *per se* increased p-STAT3 in the ARC with no further activation after LPS. Differently to the hypothalamic response, LPS increased p-STAT3 in the nucleus of the solitary tract (NTS) and raphe pallidus both in control diet and HFD fed groups, although this response was lower in the latter group. Despite not affecting food intake, LPS decreased body weight in HFD rats, which was associated with increased number of Fos and Fos/TH neurons in the NTS, and HFD-LPS showed higher number of activated noncatecholaminergic and catecholaminergic neurons in the NTS, compared with control diet-LPS group. In conclusion, our data indicate that LPS activates STAT3 mediated pathway in the hypothalamus and brainstem, leading to hypophagia, however acute effects of LPS on food intake, but not body weight loss, is abolished in HFD leptin resistant rats. Despite the absence of hypophagic effect, acute LPS under HFD induced body weight loss with higher brainstem neuron activation, suggesting that resistance to acute LPS effects under HFD might be selective to the hypothalamus.

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P737

Association of mitochondrial respiratory chain polymorphisms with obesity and type 2 diabetes in the Spanish population

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Objective

To study the association between genes codifying for mitochondrial respiratory chain (MRC) subunits and BMI and obesity as well as the impact on the risk to develop type 2 diabetes in the general population from Spain.

Research design and methods

Three thousand seven hundred and thirty-one subjects (age range 21–89) from three different population-based studies of Spain, were studied. Forty-eight single nucleotide polymorphisms (SNPs) of chromosomal genes which codify MRC proteins were selected and processed by the SNPlex method.

Results

Significant associations were observed between polymorphisms rs4600063 (SDHC gene), rs11205591 (NDUFS5 gene), rs10891319 (SDHD gene) and BMI (P value $=0.04$, 0.0011 and 0.0004 respectively) and obesity risk (OR $=0.72$, P value $=0.0072$; OR $=0.72$, $P=0.039$ and OR $=1.25$, $P=0.0038$ respectively). In addition, polymorphisms rs11205591 and rs10891319, showed a significant epistatic interaction for BMI levels and obesity risk. Finally, the GG genotype of rs11205591 polymorphism significantly reduced the risk of being diabetic after including age and sex as covariables (OR $=0.32$, 0.17–0.62; P value $=0.0001$), and BMI (OR $=0.37$, 0.19–0.72; P value $=0.0008$).

Conclusions

Polymorphisms of genes codifying MRC can be involved in BMI variation and can be related to the risk for being obese in the Spanish general population. Furthermore, the rs11205591 (NDUFS5 gene) polymorphism might contribute to the risk to develop type 2 diabetes.

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P738

The FXR agonist obeticholic acid normalizes lipid droplet and triglyceride handling in visceral adipose tissue preadipocytes from a non-genomic rabbit model of metabolic syndrome

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Liver and visceral ectopic fatty tissue disposition is a major metabolic derangement leading to type 2 diabetes, hypertension, dyslipidaemia, recapitulated in the metabolic syndrome (MetS). We have demonstrated that the selective FXR agonist obeticholic acid (OCA, INT-747) ameliorates the metabolic profile and reduces visceral adipose tissue (VAT) in a high-fat diet (HFD)-induced rabbit model of MetS. We now report the effects of OCA treatment in HFD (0.5% cholesterol and 4% peanut oil) rabbits, and on the adipogenic capacity of isolated VAT rabbit preadipocytes (rPAD). VAT and liver were studied by immunohistochemistry, Western blot and RT-PCR. Isolated rPAD were exposed to adipocyte differentiating mixture (DIM) to evaluate their adipogenic ability. Adipocyte size, as well as expression of the anti-lipolytic protein perilipin-1 and cytosolic GLUT-4, indicating adipocyte dysfunction, were significantly increased in VAT of HFD compared to regular diet (RD) rabbits, and normalized by OCA treatment. TNFα expression, along with other steatosis (PPARγ and adiponectin) and inflammation (TNFα, IL6 and IL10) markers were also significantly increased in HFD liver and normalized by OCA treatment. Interestingly, rPAD from HFD-rabbits showed a reduced responsiveness to DIM, and in particular to insulin, as demonstrated by reduced triglyceride synthesis, decreased glucose uptake, and impaired lipid droplets fusion, as well as by the reduced induction of adipogenesis- and lipid droplet-handling specific genes. OCA treatment preserved all the DIM-induced adipocyte functions, normalizing the markedly increased lipid droplet size in HFD-derived adipocytes and the increased major lipid-fusion complex SNARE. In conclusion, OCA dosing in a MetS rabbit model positively affects liver and VAT functions, increasing their efficiency in triglyceride and lipid droplet handling. This could reflect the ability of OCA to restore insulin sensitivity in committed adipose tissue unable to finalize its storage function, thus counteracting MetS-induced metabolic alterations and pathological fatty tissue disposition.

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P739

Corticotrophin realising factor affects the immune phenotype of adipocytes via CRF1 and CRF2 receptors

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Introduction
The corticotrophin-releasing factor (CRF) family of neuropeptides and their receptors affect innate immunity. A fully working peripheral CRF system is present in adipose tissue. In obesity, macrophages and adipocytes interact causing local and systemic inflammation. In addition, macrophages and adipocytes share common characteristics including production of inflammatory cytokines in response to lipopolysaccharides (LPS) via the toll-like receptor-4 (TLR4) which both macrophages and adipocytes express. Aim of this work was to examine the effects of CRF1 and CRF2 agonists and antagonists on the immune machinery of adipocytes.
Description of methods
We have used 3T3L1 mouse pre- and differentiated adipocytes. IL6/IL8 were measured by ELISA, the TLR4 receptor by RT-PCR and FACS analysis.
Results
Our data are as follows: i) LPS induced TLR4 expression and the production of interleukins IL6 and IL8 by mature adipocytes but not by pre-adipocytes. ii) LPS induced the differentiation of pre-adipocytes to adipocytes. iii) pre-adipocytes and adipocytes expressed the CRF1 and CRF2 receptors. iv) CRF1 agonists decreased basal and LPS-induced differentiation of pre-adipocytes. v) CRF2 agonists transiently suppressed the expression (transcript and protein) of TLR4 as well as the production of interleukins by pre-adipocytes. vi) both CRF receptor agonists inhibited basal and LPS-induced expression of TLR4 as well as the production of interleukins and adipokines (adiponectin and leptin) in mature adipocytes.
Conclusion
Our results indicate that CRF neuropeptides suppress TLR4 expression in adipocytes via CRF2, an effect containing their pro-inflammatory activity. They also suppress the differentiation of pre-adipocytes exclusively via CRF1. Thus, CRF appears to exert an anti-inflammatory effect in adipocytes.

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P740

Obese hypogonadal men treated with testosterone undecanoate injections up to 5 years substantially and progressively lose weight

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Introduction
Abdominal adipose tissue suppresses testosterone production by various mechanisms affecting the hypothalamic–pituitary–gonadal axis. Hypogonadism leads to further accumulation of fat mass thus creating a vicious circle. This study analysed the effects of restoring testosterone in obese hypogonadal men.
Methods
Cumulative, prospective, registry study of 181 men (mean age: 59.11 ± 6.06 years) with testosterone levels below 12.1 nmol/l and a BMI of ≥ 30 kg/m^2. All men received parenteral testosterone undecanoate 1000 mg/12 weeks following an initial 6-week interval. 89 men were treated 5 years, 114 4 years, 133 3 years, 159 2 years, 181 1 year. The changing numbers do not reflect drop-out rates but are a result of the design as new patients are added once they have received at least 1 year of treatment.
Results
At the end of the observation period, mean weight (kg) decreased from 114.71 ± 11.59 (minimum 87.0, maximum 139.00) to 93.24 ± 8.49 (min 80.0; max 115.0). This decrease was statistically significant vs baseline ($P < 0.0001$) and each year compared to previous year ($P < 0.0001$). Mean change from baseline was -16.41 ± 0.3%. After 5 years, all men had lost any weight, 99% had lost ≥ 5 kg, 90% ≥ 10 kg, 70% ≥ 15 kg, and 40% ≥ 20 kg.
Waist circumference (cm) as a measure of abdominal fat decreased from 111.2 ± 7.54 (min 89.00; max 129.00) to 100.47 ± 7.11 (min 84.00; max 117.00), BMI from 36.72 ± 3.72 (min 30.10; max 46.51) to 30.22 ± 2.6 (min 25.66; max 36.71). Fasting glucose decreased from 5.84 ± 0.84 to 5.41 ± 0.12 mmol/l, total cholesterol from 7.63 ± 0.95 to 4.9 ± 0.28, LDL from 4.47 ± 1.03 to 2.94 ± 0.93, triglycerides from 3.31 ± 0.56 to 2.17 ± 0.13 mmol/l. Systolic blood pressure decreased from 159.17 ± 15.9 to 139.08 ± 10.99 mmHg, diastolic blood pressure from 96.5 ± 11.01 to 80.39 ± 7.51 mmHg ($P < 0.0001$ for all).
Conclusion
Normalising testosterone produced loss of weight/waist circumference and improved metabolic profile. These improvements were progressive over 5 years.

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P741

Consumption of low-carbohydrate/high fat diets impairs glucose tolerance in rats independent of changes in body composition

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Moderate, 'Atkin's-style' low-carbohydrate/high fat diets (LC-HFD) are claimed to induce weight loss in overweight subjects and to ameliorate glucose metabolism, whereas extreme ketogenic LC-HFD are used to treat neurological disorders like pediatric epilepsy. To investigate the effects of both types of LC-HFD on glucose and insulin metabolism independent of the energetic intake, we pair-fed male Wistar rats isoenergetic of standard rodent chow (CH) or one of

two different LC-HF diets (percentage of metabolizable energy, fat/protein/CHO: LC-HF-1 (78.7/19.1/2.2), LC-HF-2 (92.8/5.5/1.7) and CH (16.7/19./64.3)) for 4 weeks. Since we had previously observed increased visceral fat accumulation in rats pair-fed the LC-HFD, we also studied rats in which the fat mass was clamped to the control group by restricting LC-HFD intake to 80% of caloric intake of the control group. In pair-fed groups, rats on LC-HFD displayed significantly higher fat mass when compared to CH ($P<0.01$). Furthermore, dynamic challenge tests (oGTT, i.p.GTT, insulin tolerance tests and hyperinsulinemic-euglycemic clamp) revealed that rats pair-fed the LC-HF diets have impaired glucose tolerance. As expected, the reduction of LC-HFD intake to only 80% equalized the fat mass between LC-HFD groups and controls. The restriction of LC-HFD intake to 80% improved glucose tolerance during oGTT compared to rats which were regularly pair-fed with LC-HFD. However, glucose tolerance was still impaired when compared to rats fed the standard control diet (AUC of glucose during oGTT: Chow: 12 986±801; LC-HF-1 (80%): 15 662±1111; LC-HF-2 (80%): 23 809±1485; CH vs LC-HF-1 (80%): $P=0.087$; CH vs LC-HF-2 (80%): $P<0.001$). In summary, these data clearly argue against a beneficial effect of LC-HFD on glucose and insulin metabolism. Impaired glucose tolerance occurred with LC-HFD independent of the relative abundance of fat and protein and in the absence, energy overconsumption and increased fat mass.

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P742

Depression and suicidal ideation among adults with metabolic syndrome: data from the 2008–2010 Korea national health and nutrition examination survey

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Aim
Suicide and depression are one of the highest public health problems worldwide. Suicidal ideation represents an important phase in the suicidal process and often precedes suicidal attempts or completed suicide. Patients with chronic medical disease are more likely to report suicidal thoughts and depression. However the studies on relationship between these conditions and metabolic syndrome are rare. We aimed at investigating the prevalence of depression and suicidal ideation among adults with metabolic syndrome in Korea.
Methods
We analyzed data for 17 924 persons (men 7516 persons and women 10 408 persons) from 2008–2010 KNHANES who did not have cancer or hepatitis or liver cirrhosis. Each individual was assessed for the presence of metabolic syndrome according to the NECP-ATPβ criteria except for waist circumference, for which new criteria recently suggested by Korean Society for Study of the Obesity was used. The presence of depression or suicidal ideation and were defined by a self-reported questionnaire asking if the participants had ever been diagnosed with depression by medical doctor or had any suicidal thoughts.
Results
The prevalence of depression (17 vs 14%, $P<0.001$) and suicidal ideation (20 vs 17%, $P<0.001$) was significantly higher in participants with metabolic syndrome. Mean scores for the EQ-5D decreased significantly with participants with depression (0.80±0.22 vs 0.91±0.13, $P<0.001$) and suicidal ideation (0.79±0.22 vs 0.92±0.13, $P<0.001$) in the group with metabolic syndrome.
Conclusions
This study shows that metabolic syndrome is associated with depression and suicidal ideation and this relationship was negatively associated with health related quality of life.

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P743

Early changes in glucose, insulin, C-peptide and GLP-1 levels after test meal in obese patients after gastric bypass surgery

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Introduction
Various hypothesis exist about the mechanism of changes in glucose control and insulin sensitivity after bariatric surgery.
Description of methods/design
The aim of our study was to determine glucose, insulin, C-peptide and GLP-1 levels after test meal (Fresubin drink a 200 ml given at 0 min; 200 kcal, 15% protein, 30% fat and 55% carbohydrate) before (day 0) and 5 days after gastric bypass surgery in a 22 obese patients (age: 36.22±12.66; BMI: 44.60±4.31 kg/m^2). Glycaemia (mmol/l; glucose oxidase), insulin (ECLIA, Roche Diagnostics, pmol/l), C-peptide (ECLIA, Roche Diagnostics, pmol/l) and GLP-1 (active 7–36) (pM/l; ELISA, ALPCO Diagnostics) were determined in two separate days in 0, 15, 30, 45, 60, 90 and 120 min.
Results
There were no significant difference between areas under the glucose curve (X±s.d.) (645.562±20.545 vs 621.600±24.07 mmol/l per min; $P=0.304$) and under the C-Peptide curve (293 074.125±23 539.975 vs 267 750.375±19 685.409 pmol/l per min; $P=0.317$) while there was significantly lower area under the insulin curve in day 5 (38 263.075±6079.509 vs 23 539.875±2571.388 pmol/l per min; $P=0.032$). There was significant increase in area under the GLP-1 curve in day 5 (163.00±73.61 vs 861.94±251.22 pmol/l per min; $P<0.05$).
Conclusion
In conclusion, insulin response after test meal is significantly decreased after gastric bypass surgery after 5 days without significant difference in glucose response, indicating early improvement in insulin sensitivity. The significant improvement in GLP-1 response after test meal among patients after gastric bypass surgery may be responsible for the metabolic effects of bariatric surgery, especially on glucose homeostasis.

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P744

Modulation of gene expression by 3-iodothyronamine: evidence of a lipolytic pattern

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3-Iodothyronamine (T_1AM) is a recently discovered compound which can be regarded as a novel hormone, since it is an endogenous relative of thyroid hormone with systemic distribution, which does not interact with thyroid hormone receptors, but rather with specific G protein-coupled receptors. T_1AM has been reported to modulate energy metabolism, and in rodents chronic T_1AM treatment has been associated with lipolysis and decreased body weight. To investigate the mechanism of this action we determined the effects of *in vivo* T_1AM administration on gene expression in rat adipose tissue and liver.
Eight Wistar rats were treated with T_1AM for five days (10 mg/kg per day by i.p. injection). The rats were sacrificed by guillotine and tissue samples were immediately removed and frozen in liquid nitrogen. Gene expression was evaluated by two-colour microarray analysis, using the whole rat genome G4131F microarrays (Agilent Technologies, Palo Alto, CA, USA). Significant differences in gene expression were confirmed by quantitative PCR.
In adipose tissue we detected 378 differentially expressed genes (DEGs), 268 up-regulated and 110 down-regulated, while in liver DEGs were 114 (63 up-regulated and 51 down-regulated). Functional analysis of microarray results revealed interesting interplays among DEGs. In adipose tissue pathway analysis provided evidence of decreased adipogenesis and stimulated lipolysis and fatty acid catabolism; modulated genes included acyl-CoA synthase 5, peroxisomal biogenesis factor 5, sirtuin 2, CCAAT/enhancer binding protein β (C/EBPβ) and the adiponectin receptor PAQR3. In liver most of the differentially expressed genes were of unknown function but glycerol kinase and malic enzyme were inhibited.
We conclude that *in vivo* T_1AM administration produced significant transcriptional effects, which are expected to stimulate lipid catabolism and induce a reduction of fatty mass. These actions might therefore provide the basis for the reported effectiveness of T_1AM as a lipolytic agent.

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P745

Evaluation of gastric bypass effect on cardiovascular risk and quality of life in our area

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Objectives
i) To determine the prevalence of major comorbidities of morbid obese, ii) to evaluate the gastric bypass effect on this comorbidities, on the 10 years estimated cardiovascular risk and iii) To assess the impact of bariatric surgery on the quality of life in these patients.
Methods
Cohort study with intrasubject measures (before–after) in a sample of patients with morbid obesity who underwent gastric bypass. Demographic characteristics, anthropometric parameters, cardiovascular risk factors and surgical complications were analyzed. The estimation of cardiovascular disease risk at 10 years was determined according to the Framingham Risk Score and the impact on the quality of life using the BAROS test (Bariatric Analysis and Reporting Outcome System).
Results
162 patients were included (74.3% females), with mean age 38.87 ± 10.11 years and BMI before surgery 51.12 ± 7.22 kg/m^2. Two years after surgery the percentage of weight lost was 72.85%. Four months after the bypass only 12 of the 41 patients with type 2 diabetes maintained the diagnosis of diabetes. Two years after surgery, the resolution of hypertension, dyslipidemia and diabetes occurred in 71.93, 92.7, and 92.68% cases respectively ($P<0.001$). According to the Framingham Risk Score, 22.7% presented with a risk greater than 10% before surgery. Mean risk decreased from 5.82% at baseline to 2.21% 2 years after surgery ($P<0.001$). 14.9% of patients had early complications and 27.2% developed later complications (the most frequent eventration). BAROS scale was excellent in 36.8% of cases, very good in 36.7% and good in 21.1% at 2 years.
Conclusions
Gastric bypass is an effective tool in weight loss, early beneficial effects on metabolic disorders, reduction in cardiovascular risk and improvement quality of life in morbid obese patients in our area.

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P746

Green tea catechins attenuate high-fat diet effects related to obesity and diabetes without effect hypothalamic expression of TLR4 pathway or serotoninergic 1B and 2C receptors

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Many mechanisms are proposed for green tea's role as anti-obesity and anti-diabetic natural product. Two of them involve the hypothalamic control of energy balance: i) high-fat diets (HFD) rich in saturated fats induce low-grade inflammation, leading to leptin and insulin resistance; ii) serotonin receptors (SR) 1B and 2C activation have anorexigenic effects, SR2C activation improves insulin sensitivity, and HFD can also impair this system. We investigated the effects of green tea catechins (50 mg/kg, gavage) on metabolic effects of 60 days treatment of HFD (30% lard) in male Swiss mice. Groups: control diet + tap water (C) or catechins (CE); HFD + tap water (H) or catechins (HE). Glucose tolerance test (GTT) was performed 3 days before sacrifice. After overnight fasting, mice were sacrificed and serum was analyzed for glucose, HDL-cholesterol, total cholesterol, triglycerides, adiponectin, and insulin. Besides, we investigated whether these metabolic events were related to the hypothalamic inflammatory pathway (TLR4) and/or serotoninergic system (SR1B and SR2C). H mice presented increased GTT area under curve and higher fat pads (sum of epididymal, retroperitoneal and mesenteric). Green tea catechins attenuated the increased GTT area under curve ($F=9.3$ (3.37); $P=0.08$ H vs HE) without effects on fat pads ($F=7.9$ (3.86); $P<0.05$ vs C). Fasting serum glucose, total cholesterol, and triglycerides were not different among groups. H mice presented lower HDL-cholesterol ($F=7.9$ (3.31); $P<0.05$ vs C, E, and HE), higher HOMA-IR ($F=9.3$ (3.27); $P<0.05$ vs C), and higher insulin ($F=7.2$ (1.14); $P<0.05$ vs C), which were improved by green tea catechins. Expression of TLR4 pathway proteins, SR1B and SR2C in the hypothalamus was not different among treatments. In conclusion, green tea catechins prevented some of the peripheral negative effects of HFD, which were not related to the expression of analyzed hypothalamic proteins. Whether other proteins of the inflammatory pathway or serotoninergic system are involved is to be investigated.

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P747

Short-term ovariectomy did not cause tissues proinflammatory state, but associated with hyperlipidic diet caused hyperleptinemia and increased body weight gain

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Obesity leads to low-grade inflammation, and high-fat diet is one of the major causes. On the other hand, menopause can aggravate it by promoting food intake alterations, and metabolic and endocrine adaptations. Thus, we used ovariectomized rats fed with high-fat diet to evaluate changes on the tissue inflammatory state. Forty days-old female Wistar rats were fed with: a hyperlipidic diet enriched with lard – H-group; or chow diet – C-group; for 30 days. After this period, the H and C were divided in four groups: sham-operated (SH and SC), ovariectomized (OVXH and OVXC). The respective diets were maintained, and 30 days after the surgery, the animals were euthanatized. Serum, mesenteric adipose tissue, gastrocnemius muscle and liver were collected. Tissues IL10, TNFα and IL6 level, body weight gain, serum lipid profile, leptin and insulin were determined. MANOVA was used for statistical analysis, with Scheffé's *post-hoc* test and $P<0.05$. All parameters analysed were similar between SC and OVXC groups. The OVXH had higher mesenteric adipose tissue weight than SH ($F=4.9$ (3.27); $P<0.05$). The high-fat diet caused a decrease in IL10, IL6 and TNFα in mesenteric adipose tissue from SH and OVXH rats compared to control diet. The association between hyperlipidic diet and ovariectomy promoted an increase in total body weight gain ($F=8.5$ (3.27); $P<0.05$) and serum leptin ($F=8.3$ (3.27); $P<0.05$) level in relation to others groups. These results demonstrate that 30 days of ovariectomy did not affect the analysed parameters. However, hyperlipidic diet associated to ovariectomy increases body weight gain and leptinemia. These results suggest that short period without oestrogen was not able to promote proinflammatory state in the assessed tissues, also that the hyperlipidic diet could potentiate the effects of ovariectomy on obesity development with hyperleptinemia.

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P748

Differences among clinical, analytical and psychological outcomes among patients with an eating disorder after bariatric surgery

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Introduction
Information regarding eating disorders (ED) in postsurgical outcomes after bariatric surgery (BS) is scarce.
Objectives
To analyze the effect of BS on ED and to investigate if ED influence clinical, biochemical or psychological outcomes.
Methods
Sixty patients (78.3%♀, age 46.35 ± 9.89, months since BS 46.28 ± 28.1) who underwent BS, with a minimum follow up of 12 months, were evaluated cross-sectionally. Initial and current BMI, depressive symptoms, comorbidity, sociodemographic and biochemical parameters were recorded. For the screening

of ED, QEWP-R was administered.
Results
Before BS, five subjects were diagnosed of ED, two binge eating disorders (BED), one bulimia nervosa (BN) and one eating disorder non otherwise specified (EDNOS). After BS, BED resolved in all patients, BN persisted and EDNOS progressed to BN. Furthermore, after BS, 13 new cases of BED (21.6%) and six cases of BN (10%) were detected. Time from surgery was higher among BED patients (61.62 $\pm$ 23.47 vs 38.8 $\pm$ 26.44 months; $P=0.022$); Furthermore, in these patients a greater proportion of calories obtained from alcohol intake (3.61 $\pm$ 6 vs 0.65 $\pm$ 1.74%; $P=0.041$), a more prevalent history of prebariatric psychiatric disorders (85 vs 51.2%; $P=0.034$) and a higher proportion of subjects who regained weight (61.5 vs 26.8%; $P=0.024$) was observed. BN subjects had greater depression scores (17 $\pm$ 4 vs 8.1 $\pm$ 5; p = 0.04). Both entities had more episodes of vomiting (61.5 vs 29.3%; $P=0.048$ and 83.3 vs 61.5%; $P=0.047$). Subjects with ED had more difficulties in following visits after BS (19.5 vs 53.8%; $P=0.028$ and 19.5 vs 66.7%; $P=0.01$).
Conclusions
Development of ED is frequent after BS. Owing to its potential association with weight regain, systematic screening of ED after BS is warranted.

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P749

BMI as a prognostic feature in patients with breast cancer treated with chemo/endocrine therapy

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Introduction
High BMI has been associated with an increased risk for breast cancer among premenopausal and postmenopausal women. Several biological mechanisms play a significant role in the genesis and progression of breast cancer.
Material and methods
This study aimed to investigate relationship between BMI and breast cancer diagnosis or progression in a Croatian population. BMI, presence or absence of breast cancer and its clinical-pathological characteristics were analyzed in a series of 110 breast cancer women and compared with those of 110 healthy women prospectively.
Results
BMI was significantly associated with a larger-tumour size (BMI $\geq$30 respect to normal weight, $P=0.0047$) and a higher probability of having positive axillary lymph node ($P<0.0001$).
Conclusion
The analysis of pathological features of cancer indicates that normal weight women have a significantly higher probability of having a smaller breast cancer at time of diagnosis and negative axillary lymph nodes. BMI is an independent prognostic factor in patients with breast cancer treated with chemo/endocrine therapy in Croatian population.

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P750

Osteocalcin: more than a bone marker: the Odense Androgen Study

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Introduction
The osteoblast derived bone formation marker osteocalcin has been reported to decrease adipose tissue in mice. In elderly men, osteocalcin was reported to correlate inversely with fat mass.
Aim
To examine the relationship between osteocalcin and regional fat depots.
Method
The Odense Androgen Study is a population-based, cross-sectional study of 779 randomly selected men aged 20–29 years. Total, central, upper extremity, and lower extremity fat mass (TFM, CFM, UEFM, and LEFM) was assessed by DXA. Abdominal subcutaneous, thigh subcutaneous, visceral, and thigh intramuscular adipose tissue (abdSAT, thiSAT, VAT, and IMAT) was assessed by MRI. Osteocalcin, bone-specific alkaline phosphatase (bsALP), carboxy-terminal telopeptide of type 1 collagen (1CTP), insulin, glucose, and lipids, were analyzed in serum samples drawn in fasting state between 0800 and 1000 h Blood pressure was recorded.
Results
Osteocalcin correlated negatively with TFM ($R=-0.18$, $P<0001$), CFM ($R=-0.21$, $P<0.0001$), UEFM ($R=-0.18$, $P<0.0001$), LEFM ($R=-0.12$, $P=0.001$), abdSAT ($R=-0.18$, $P=0.0003$), VAT ($R=-0.27$, $P<0.00001$), and IMAT ($R=-0.16$, $P=0.001$). No correlations were observed between the fat parameters and the other bone markers despite highly significant correlations between osteocalcin and bsALP ($R=0.40$, $P<10^9$) and 1CTP ($R=0.41$, $P<10^9$). Osteocalcin decreased from age 20 to 29 years ($R=-0.40$, $P<10^9$), while CFM ($R=0.14$, $P=0.0001$), abdSAT ($R=0.12$, $P=0.02$), VAT ($R=0.25$, $P<0.0001$), and IMAT ($R=0.11$, $P=0.03$) increased significantly. Osteocalcin correlated negatively with total cholesterol ($R=-0.09$, $P=0.01$), LDL-cholesterol ($R=-0.09$, $P=0.009$) and diastolic blood pressure ($R=-0.07$, $P=0.046$).
Conclusion
In cross-sectional analyses, osteocalcin was the only bone marker that correlated with adiposity: osteocalcin correlated inversely with all fat parameters, except thiSAT. The age-related decline of osteocalcin was accompanied by an increase in deep adipose tissues (VAT rather than abdSAT, IMAT rather than thiSAT). High blood pressure and dyslipidemia was associated with low osteocalcin levels. Our data support the hypothesis that osteocalcin is negatively associated with adiposity in humans.

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P751

Associations between smoking and components of the metabolic syndrome

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Background
The clustering of metabolic and cardiovascular risk factors is known as the metabolic syndrome (MetS). Lifestyle factors like smoking may contribute to the differences in prevalence of the MetS. The aim of this study was to examine the association between smoking and the MetS and its components in different BMI classes.
Methods
The present cross-sectional evaluation comprised 24 762 men and 35 558 women, participating in the LifeLines Cohort Study, of whom 6,058 and 7,469 were current smokers. MetS was defined using the criteria of the NCEP ATP III. Participants were categorized in different BMI classes (i) BMI <25; ii) BMI 25–30; iii) BMI >30 kg/m^2). Linear regression was used to test the association between smoking and the components of MetS, stratified by gender and age.
Results
There was an increasing prevalence of MetS with increasing BMI. In obese men (BMI ≥ 30 kg/m^2) 62% fulfilled the MetS criteria, for women this was 41%. Overall, current smoking was associated with increased risk for MetS in both genders and all BMI classes (OR's 1.7–2.4 for men, 1.8–2.3 for women, all $P<0.001$). In all BMI classes, there was a dose-dependent association between the amount of tobacco smoked daily and the prevalence of MetS. Compared to non-smokers, current smokers had lower levels of HDL cholesterol, and higher levels of triglycerides and waist circumference in all BMI-classes (all $P<0.001$). Smoking had no consistent association with blood pressure or fasting blood glucose levels. We observed a dose-dependent association between the amount of tobacco smoked daily and lower HDL cholesterol and higher triglyceride levels in normal weight, overweight and obese smokers (all $P<0.001$).
Conclusions
Smoking is associated with an increased risk for the metabolic syndrome, in all BMI classes. This increased risk was mainly related to lower levels of HDL cholesterol, and increased triglycerides and waist circumference.

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P752

Monocyte chemoattractant protein-1 is associated with apolipoprotein A-I and apolipoprotein e levels in obese premenopausal women

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Background
Obesity represents a cardiovascular risk factor. Monocyte chemoattractant protein-1 (MCP-1) is a known regulator of monocyte recruitment to sites of inflammation, and participates at the early phases of atherogenesis. Apolipoproteins, as part of the lipoprotein particles, are classically coupled with cardiovascular risk distinction.
Aims
To assess the association of anthropometric parameters, insulin resistance and MCP-1 levels with classic lipid and apolipoprotein profile, in both obese and normal-weight premenopausal women.
Methods
We studied 72 obese (age = 34.6 ± 7.8 years, BMI = 44.4 ± 8.5 kg/m^2, waist circumference = 118.6 ± 15.1 cm) and 73 normal-weight pre-menopausal women (age = 33.6 ± 8.4 years, BMI = 21.4 ± 1.7 kg/m^2, waist circumference = 71.5 ± 5.8 cm). Women were characterized for anthropometrics and a fasting blood sample was collected for MCP-1, insulin, glucose, total, LDL and HDL-cholesterol, triglycerides, free fatty acids and apolipoproteins A-I, A-II, B, C-II, C-III and E; insulin resistance was assessed by the homeostatic model assessment (HOMA-IR). We compared biochemical parameters between groups. In each group, we looked for correlations of lipid/apolipoprotein profile with MCP-1, insulin resistance and anthropometrics.
Results
Obese women presented significantly higher triglycerides ($P < 0.001$) and MCP-1 levels ($P = 0.001$) and significantly lower levels of HDL-cholesterol ($P < 0.001$) and apolipoprotein A-I ($P < 0.001$); we also found a trend for lower apolipoprotein A-II ($P = 0.07$). In the obese group (but not in the non-obese), MCP-1 was directly correlated with apolipoprotein E ($P = 0.007$; $r = 0.35$) and inversely associated with HDL-cholesterol ($P = 0.013$; $r = -0.321$) and apolipoprotein A-I ($P = 0.021$; $r = -0.3$). HOMA-IR was inversely associated with HDL-cholesterol ($P = 0.032$; $r = -0.252$) and directly associated with triglycerides ($P = 0.003$; $r = 0.344$). MCP-1 was not correlated with anthropometrics or HOMA-IR.
Conclusions
The higher levels of MCP-1 and triglycerides and lower levels of HDL-cholesterol/apolipoprotein A-I observed in the obese women are consistent with a higher atherosclerotic activity. The association of MCP-1 levels with apolipoproteins A-I and E in obese women, independently from anthropometrics or insulin resistance, may indicate a way of regulation of the atherosclerotic activity in obesity.

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P753

Chronic central administration of amylin decreases fat mass but does not modify body weight

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Aims (objectives)
Energy intake and expenditure are regulated by different signals. One of the molecules implicated is islet amyloid polypeptide (IAPP or amylin), one of the major secretory products of β-cells of the pancreatic islets of Langerhans. It is a peptide that inhibits insulin and glucagon secretion, and can also act through the brain decreasing food intake and inhibiting gastric emptying. In this study, we investigated the potential effects of the chronic central administration of amylin on energy homeostasis.
Materials and methods
Male Sprague-Dawley rats were administered chronically with amylin or saline by a mini-osmotic pump connected to an intracerebroventricular cannula. Rats were monitored in the indirect calorimetry system and several parameters (O_2 consumption, CO_2 production, daily intake, body weight…) were assessed.
Results
Chronic central administration of amylin did not cause significant differences in body weight, energy expenditure, locomotor activity and respiratory quotient. However, there is a greater loss of fat in rats treated with amylin respect saline.
Conclusions
Chronic central infusion of amylin only caused a slight decrease in fat mass, without any difference in any of the measured parameters.

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P754

Apelin levels in men with metabolic syndrome with or without late-onset hypogonadism

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Apelin is a new adipokine associated with obesity. Data about the relationship of apelin to the metabolic syndrome (MS) are still scarce. Late-onset hypogonadism (LOH) is common in men with MS, but we did not find data about the levels of apelin in men with LOH.
The aim of this study was to determine the levels of apelin in men with MS with or without LOH.
Patients and methods
Ninety nine men were included in the study. Of them 65 had MS (IDF 2005) and they were devided according to their morning total testosterone (TT) level (cutoff 10.4 nmol/l) into two groups: MS-LOH ($n = 21$) and MS-NoLOH ($n = 44$). The control group consisted of 34 men without MS and LOH. Apelin was determined in serum using enzymelinked immunosorbent assay.
Results
MS men were at mean age (± S.D.) = 50.4 ± 9.6 years; BMI = 33.3 ± 7.7 kg/m^2; waist circumference (WC) = 111.7 ± 13.9 cm and TT = 13.6 ± 5.4 nmol/l. The control group was at age = 51.5 ± 6.4 years (NS); BMI = 25.7 ± 2.4 kg/m^2 ($P < 0.001$); WC = 89.8 ± 8.2 cm ($P < 0.001$) and TT = 17.9 ± 5.6 nmol/l ($P < 0.001$). MS-LOH sub-group had age 47.9 ± 10.5 years; BMI = 36.4 ± 9.5 kg/m^2; WC = 114.6 ± 16.7 cm; TT = 8.1 ± 1.5 nmol/l and MS-NoLOH sub-group – age 51.6 ± 9.1 years (NS); BMI = 31.8 ± 6.3 kg/m^2 ($P < 0.05$); WC = 110.4 ± 12.5 cm (NS); TT = 16.3 ± 4.5 nmol/l ($P < 0.001$). The levels of apelin were higher in the MS group – 1.61 ± 0.53 ng/ml compared to the control one – 1.38 ± 0.57 ng/ml ($P < 0.05$). There was no difference between MS-LOH – 1.53 ± 0.52 ng/ml and MS-NoLOH – 1.65 ± 0.53 ng/ml sub-groups. The MS-NoLOH differed from the control group ($P < 0.05$).
Conclusions
In this study higher apelin levels were found in the presence of MS compared to healthy men, but did not differ between men having MS with or without LOH.

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P755

Apnoeic episodes impair insulin resistance independently of oxygen desaturations: evidence from morbid obese patients with discordant apnoea/desaturation indexes

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Morbid obesity (MO) is often complicated by obstructive sleep apnoea (OSA), which in some cases is evaluated by pulsioximetry. This cross-sectional study was designed to investigate the prevalence of discordance between desaturation index (DI) and apnoea–hypopnoea index (AHI) and its effects on insulin resistance and secretion in MO patients candidate to bariatric surgery ($n = 496$) in whom a full polysomnographic study was carried out. We identified 163 (33%) out of 496 patients with normal DI (< 3/h), 42 (26%) of which showed significant nocturnal apnoeas (AHI > 10). When comparing DI less than three patients with IAH > 10 ($n = 42$) with those showing IAH < 10 ($n = 121$), apnoeic subjects showed higher fasting insulin (17.4 + 1.1 vs 15 + 0.6 mU/l, $P < 0.05$), post-OGTT insulin peak (151.2 + 11.5 vs 123.2 + 5.6 mU/l, $P < 0.05$) and HOMAr values (4.4 + 0.3 vs 3.6 + 0.1, $P < 0.05$) than those with AHI < 10, despite exhibiting similar waist and neck circumference. On the other hand, there were 333 patients with DI > 3, of which 60 (18%) showed AHI < 10. In this subgroup of patients with significant

oxygen desaturations there were no differences in fasting, post OGTT insulin levels or HOMAr between patients classified according with AHI values. No differences were seen in insulinogenic index between patients with AHI >10 and AHI <10 in any of DI subgroups. AHI values were correlated with peak insulin values after OGTT ($r=0.15$, $P<0.01$), QUICKI ($r=-0.17$, $P<0.01$), HOMAr ($r=0.14$, $P<0.05$) and Matsuda index ($r=-0.10$, $P<0.01$) only in patients with DI >3. These findings show that discordant results between AHI and DI can be observed in up to 26% of cases when the diagnosis is based only on pulsioximetry results, leading to patient misclassification. Presence of apnoeas in absence of DI abnormalities is associated with disturbances in insulin resistance parameters, suggesting that apnoeic episodes, independently of oxygen desaturations, participate in the impairment of carbohydrate metabolism seen in MO patients.

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P756

Circulating endocannabinoids are differentially modulated during the oral glucose tolerance test

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The endocannabinoid system (ECS) is involved in the regulation of food intake and energy expenditure. Increased level of EC lipid mediators anandamide (AEA) and 2-arachydonoylglycerol (2AG) and/or a higher expression of cannabinoid receptor type 1 in central and peripheral districts are supposed to contribute to the development and maintenance of obesity and related dismetabolism. To understand the ECS response to insulin signal and to anthropometric and biochemical parameters, we evaluated the effect of an OGTT on circulating ECs in five fasted obese females (age: 41.4 ± 3.6 years, BMI: 32.5 ± 4.2 kg/m^2, waist circumference: 103.4 ± 11.1 cm). Blood samples were collected before and after 30, 60, 90 and 120 min from a 75 g glucose ingestion. Plasma AEA, related *N*-acylethanolamines palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), 2AG and 1AG were measured by LC–MS/MS. A whole body and specific region composition analysis was performed by dual-energy X-ray absorptiometry. Basal insulin and glucose levels were 9.4 ± 1.1 μU/ml and 91.0 ± 5.4 mg/dl, and the calculated area under curve (AUC) were 9.777 ± 2.601 and $15\,375\pm4.044$ respectively. AEA, PEA, OEA, 2AG and 1AG basal levels were 1.280 ± 0.498, 15.48 ± 3.70, 4.244 ± 1.450, 1.498 ± 0.465 and 0.588 ± 0.192 pmol/ml respectively. AEA, PEA and OEA significantly decreased along the OGTT ($P=0.004$, $P=0.001$ and $P=0.003$ respectively). At 60 min their level ($\Delta(t_0-t_{60})/t_{0\%}$) reduced to 0.639 ± 0.340 (51.4%), 9.16 ± 3.69 (41.2%) and 2.276 ± 0.906 pmol/ml (44.9%). Conversely, 2AG and 1AG levels did not significantly change. AEA, PEA and OEA reduction ($\Delta(t_0-t_{60})/t_{0\%}$) negatively correlated with glucose AUC ($r=-0.895$, $P=0.040$; $r=-0.929$, $P=0.022$; $r=-0.948$, $P=0.014$ respectively) and positively with whole body ($r=0.882$, $P=0.048$; $r=0.910$, $P=0.032$; $r=0.944$, $P=0.016$ respectively) and gynoid lean mass ($r=0.957$, $P=0.010$; $r=0.967$, $P=0.007$; $r=0.951$, $P=0.013$ respectively). No significant correlations were observed for BMI, waist circumference, basal glucose and insulin, insulin AUC and blood lipids. Our preliminary data indicated that *N*-acylethanolamine levels are suppressed during the OGTT, and that the extent of the suppression is promoted by lean mass and affected by increasing glucose AUC.

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P757

Adiponectin and leptin actions on DNA synthesis and cell death of porcine myoblasts are dependent on the cellular milieu and related to ERK1/2 signalling

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Cross-talk between adipose tissue and skeletal muscle could be mediated in part by adipokines secreted from adipose tissue. Previously, we observed that adiponectin but not leptin decreased DNA synthesis rate in proliferating porcine myoblasts in growth factor-supplemented serum-free medium (GF-SFM) after 48-h exposure (Will *et al.* 2012). To further elucidate the effects of adiponectin and leptin on muscle cell growth and their mode of action, this study was conducted to investigate the effects of these adipokines on cell number (DNA), DNA synthesis rate, and cell death as well as on the activation of key signalling molecules in proliferating porcine myoblasts either grown in low-serum (1% FBS) medium (LSM) or GF-SFM.

Recombinant porcine adiponectin (40 μg/ml) and leptin (20 ng/ml) supplemented to LSM increased DNA synthesis rate measured as (^{3}H)-thymidine incorporation ($P<0.01$), probably related to increased DNA repair, and reduced cell viability in terms of lactate dehydrogenase release ($P<0.05$) and/or lowered DNA content after 24-h ($P<0.05$) but not 48-h exposure. Both adiponectin ($P=0.07$) and leptin ($P<0.05$) treatment resulted in an activation of ERK1/2 (p44-42) after 15 min followed by decreased activation after 60 and 180 min ($P<0.05$). Adiponectin tended to increase c-fos activation ($P=0.08$) and to decrease p53 activation at 180 min ($P<0.05$). In GF-SFM, in contrast, adiponectin and leptin treatment decreased DNA synthesis as early as after 4-h exposure ($P<0.01$) and diminished the rate of cell death after 48 h ($P<0.05$). Under these conditions, ERK1/2 activation was reduced ($P<0.01$) after 15- and 30-min treatment with adiponectin or leptin.

In conclusion, the effects of adiponectin and leptin on the growth of porcine myoblasts are dependent on the surrounding cellular milieu and related to ERK1/2 signalling. The presence of growth factors in culture medium seems to attenuate adverse effects of the adipokines on the growth of myoblasts.

Keywords
Adiponectin, Leptin, Skeletal muscle, Pig, Satellite cell culture, ERK1/2.

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P758

Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a meta-analytic study

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Introduction

Few randomised clinical studies have evaluated the impact of diet and physical activity on testosterone levels in obese men with conflicting results. Conversely, studies on bariatric surgery in men generally showed an increase in testosterone levels. The aim of the present study is to perform a systematic review and meta-analysis of available trials on the effect of body weight loss on sex hormones levels.

Methods

An extensive Medline search was performed including the following words 'testosterone', 'diet', 'weight loss' and 'bariatric surgery' and 'males'. The search was restricted to data from January 1, 1969 up to August 31, 2012.

Results

Out of 266 retrieved articles, 24 were included in the study. Of the latter, 22 evaluated the effect of diet or of bariatric surgery, whereas two compared diet and bariatric surgery. Overall both low calorie diet and bariatric surgery are associated with a significant ($P<0.0001$) increase plasma sex hormone binding globulin bound and unbound testosterone levels (TT), bariatric surgery being more effective in comparison with low calorie diet (TT increase $=8.73$ (6.51–10.95) vs 2.87 (1.68–4.07) for bariatric surgery and low calorie diet, respectively; both $P<0.0001$ vs baseline). Androgen rise is greater in those patients that lose more weight as well as in younger, non-diabetic subjects with a greater degree of obesity. Body weight loss is also associated with and decrease in estradiol and increase in gonadotropins levels. Multiple regression analysis shows that the degree of body weight loss is the best determinant of TT rise ($B=2.50\pm0.98$; $P=0.029$).

Conclusions

Present data show that weight loss is associated with an increase of both bound and unbound testosterone levels. The normalization of sex hormones induced by body weight loss is a possible mechanism contributing to the beneficial effects of surgery in morbid obesity.

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P759

Serum levels of fetuin A and 8-hydroxydeoxyguanosine in morbidly obese subjects

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Insulin resistance is one of the feature of obesity. Fetuin A is inhibitor of insulin receptor which belongs the family of receptor tyrosine kinase. It has been observed that fetuin-null mice are resistant to diet-induced obesity and they exhibit increased insulin sensitivity. Increased production of reactive oxygen species is suggested to be associated with insulin resistance. Attacks of reactive oxygen species to DNA results in base oxidation. Among the oxidized bases, 8-hydroxydeoxyguanosine is predominant lesion with pro-mutagenic potential. In the present study; measurement of serum levels of fetuin A and 8-hydroxydeoxyguanosine in obese subjects ($n=46$) and healthy controls ($n=22$), and examination of the relations between these parameters and insulin resistance have been purposed. Blood samples were taken form morbidly obese subjects after a 12 h fasting. Serum levels of fetuin A and 8-hydroxydeoxyguanosine were measured by ELISA. Statistical analysis was performed by Mann–Whitney *U* test and correlations were examined by Spearman's correlation coefficient. Serum levels of total cholesterol, HDL, LDL, VLDL, triglycerides, T_3, T_4, fasting glucose, C-peptide and %HbA1c in the obese group were found to be different from those in the control group. Serum level of fetuin A was found to be higher, 8-hydroxydeoxyguanosine level was found to be lower in the morbid obese group than those in the control group. Fetuin A was found to be positively correlated with HOMA-IR and negatively correlated with 8-hydroxydeoxyguanosine. No significant association was determined between body mass index and measured parameters. In conclusion, serum level of fetuin A is high in morbidly obese subjects and is negatively associated with 8-hydroxydeoxyguanosine level in peripheral circulation. Fetuin A may be a promising link between insulin resistance and obesity as well its comorbidities.

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P760

Off-label GLP-1 agonist treatment in 43 non-diabetic patients: are weight loss and treatment tolerance equally promising outside of clinical trials?

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Context
Recent data from controlled clinical trials have demonstrated that GLP-1 agonists are a well-tolerated therapy-option for weight loss in obese patients without type 2 diabetes mellitus.
Objective
To investigate whether continuation of treatment, side-effects and effect on weight loss of GLP-1 agonists are equally promising in dairy clinical practice settings in non-diabetics.
Methods
Obese, non-diabetic patients of our interdisciplinary obesity centre were treated off-label with GLP-1 agonists. Application was started low dose and increased if side effects were tolerable. Monthly costs were 125 Euros for daily applications of 1.2 mg Liraglutide or 10 mg Exenatide twice daily. Data were obtained by telephone interviews about base-line characteristics, weight loss, sensation of satiation, duration of therapy, side-effects and reasons for discontinuation.
Results
Of 43 included cases (five males, mean age 43 ± 11 years, mean weight 107 ± 24 kg, mean excess weight 35 ± 21 kg), seven were treated with Exenatide and 36 with Liraglutide. Excess weight loss in linear regression models was 6.7% per months under control of age, sex, initial weight and type of GLP-1-analogon treatment and did not significantly differ between Liraglutide and Exenatide. 58% of patients reported side-effects mostly concerning the gastrointestinal tract. Surprisingly no patient reported vomiting. Two severe side-effects (pancreatitis and first manifestation of systemic lupus erythematodes) occurred. At time of telephone interview only 30.2% were continuing treatment. Mean treatment duration was 2.98 ± 2.71 months. Common reasons for discontinuation of treatment were no/little effect on weight loss (27.9%), intolerable side-effects (20.9%) or financial reasons (14%).
Conclusion
GLP 1 agonist treatment in obese non-diabetics also correlate with significant weight loss in clinical practice. However side-effects and discontinuation of treatment are common. Therefore long-term effect on weight-loss might not be as promising as suggested by data from clinical trials.

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P761

SHBG and testosterone are associated with inflammation in obese men

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Introduction
It was showed that low SHBG represent an independent bio-marker of proinflammatory states associated with insulin resistance. Recent studies provide additional evidence that insulin plays no role in decreasing hepatic SHBG production, and that proinflammatory cytokines, contribute indirectly to reducing SHBG expression in liver of obese persons by reducing hepatic HNF-4α. Obesity in men is commonly accompanied by a decline of serum total testosterone and bioavailable testosterone (BT) levels. Metabolic syndrome (MetS) is associated with a further decline in testosterone, independently of BMI and SHBG, with dose-response relationship between decreasing levels of testosterone. Previous reports suggested hypothesis that androgens may have an anti- inflammatory effect. The aim of the study was to examine the association between testosterone and SHBG to C-reactive protein (CRP) and insulin resistance in obese man.
Description of methods/design
We investigated 52 obese man mean age: 38.91 ± 12.56 years; mean BMI: 46.11 ± 10.37 kg/m^2) and measured SHBG, testosterone, CRP, fasting plasma glucose, fasting plasma insulin, HOMA-IR and anthropometric measurements (BMI, WC, WHR).
Results
SHBG was significantly inversely correlated with CRP ($r=-0.615$, $P<0.001$) and fasting insulin ($r=-0.291$, $P<0.05$). There was no statistically significant correlation between SHBG and fasting plasma glucose, HOMA-IR and BMI< WC, WHR. Levels of testosterone were inversely correlated with CRP ($r=-0.341$, $P<0.05$), BMI ($r=-0.461$, $P<0.001$) and WC ($r=-0.389$, $P<0.001$). There was no statistically significant correlation between testosterone and fasting insulin, fasting glucose, HOMA-IRr or WHR.
Conclusion
Our result suggested association of low levels of testosterone and SHGB with increased inflammation in obese man.

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P762

The effect of rutin on homocysteine induced proliferation of 3T3-L1 preadipocyte *in vitro*

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Introduction
Rutin ($C_{27}H_{30}O_{16}$) is the major representative of the flavonol subclass of flavonoids. Rutin has wide variety of pharmacological activity, however there are not sufficient data in 3T3-L1 preadipocyte. In this study, we demonstrated that the effect of rutin on the proliferation of 3T3-L1 preadipocyte.
Methods
The 3T3-L1 preadipocyte were treated with homocysteine (1 mM), rutin (30 μM) and rutin after homocysteine. And we analyzed the cell proliferation with

methylthiazoletetrazolium (MTT) assay. We evaluated the proliferation pathway and apoptotic pathway with Western blot.
Results
The proliferation of 3T3-L1 preadipocyte was enhanced with homocysteine ($P<0.001$). The cell viability of 3T3-L1 preadipocyte was not changed with rutin in diverse doses (5, 10, 30, 60 μM). But rutin after homocysteine treatment significantly suppressed the proliferation of 3T3-L1 preadipocyte compared to homocysteine treatment. In Western blot analysis, Immunoexpression of phospho-p44/42 MAPK (ERK1/2) and phospho-MEK1/2 were significantly increased by homocysteine (1 mM). However, these were suppressed with PD98059 treatment, not by wortmannin treatment.
Conclusion
These resuls suggested rutin suppressed the proliferation of 3T3-L1 preadipocyte by homocysteine through p44/42 MAPK pathway.

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P763

Preoperative metabolic status is associated with adiponectin levels attained 6 months after laparoscopic sleeve gastrectomy, independently of the degree of weight loss

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Introduction
Adiponectin is an adipocyte-derived hormone with an important role in regulation of glucose and lipid metabolism. Its variation might mediate an ameliorated metabolic profile associated with weight loss after bariatric surgery. The aim of our study was to evaluate adiponectin change as early as 6 months after laparoscopic sleeve gastrectomy (LSG) and to indicate preoperative factors that might influence its dynamic.
Methods
A number of 88 extremely obese women (mean age $=42.1\pm11.24$ years, mean BMI $=45.77\pm7.6$ kg/m^2) were evaluated before and 6 months after LSG. This included complete clinical examination, as well as metabolic and hormonal lab tests. Serum adiponectin was measured using ELISA method, while for C-reactive protein (CRP), IGF1 and insulin we used chemiluminiscence. Since IGF1 levels are age-dependent, we calculated the SDS of IGF1 levels according to age (z-score). Parametric variables are presented as mean $\pm$ S.D., while non-parametric ones (such as adiponectin) as median (interquartile range).
Results
Six months after LSG, mean BMI decreased from 45.77 ± 7.6 to 33.24 ± 7.1 kg/m^2, $P<0.001$, while adiponectin levels increased from 7.76 (3.74) to 10.4 (6.37) mg/l, $P<0.001$. Postoperative adiponectin levels negatively correlated with preoperative triglyceride ($r=-0.294$, $P=0.008$), HOMA-IR ($r=-0.326$, $P=0.005$), and CRP ($r=-0.337$, $P=0.004$) levels and positively correlated with preoperative adiponectin levels ($r=0.442$, $P<0.001$) and IGF1 z-score ($r=0.245$, $P=0.029$). There was also a positive correlation between postoperative adiponectin levels and the percentage of weight loss ($r=0.318$, $P=0.004$). In multivariate analysis, stepwise method, which included all the aforementioned variables, preoperative adiponectin and HOMA-IR levels and the percentage of weight loss remained independently associated with postoperative adiponectin levels, together explaining 52.7% of its variability.
Conclusion
Preoperative metabolic status (appreciated by adiponectin and HOMA-IR levels) and the percentage of weight loss are independent determinants of adiponectin levels attained 6 months after LSG.

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P764

Evaluation of gastric bypass effect on cardiovascular risk and quality of life in type 2 diabetes patients

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Objective
To evaluate the gastric bypass effect in type 2 diabetic patients with morbid obesity, on the following terms: diabetes evolution, 10 years estimated cardiovascular risk and quality of life
Methods
Cohort study with intrasubject measures (before–after) in a sample of patients with morbid obesity and type 2 diabetes who underwent gastric bypass. Demographic characteristics, anthropometric parameters, cardiovascular risk factors and surgical complications were analyzed. The estimation of cardiovascular disease risk at 10 years was determined according to the Framingham Risk Score and the impact on the quality of life using the BAROS test (Bariatric Analysis and Reporting Outcome System).
Results
41 patients were included (63.4% female), with mean age 45.62 ± 8.37 years and BMI before surgery 51.96 ± 8.69 kg/m^2. In most cases duration of diabetes was <10 years, 92.6% used oral hypoglycemic therapy and with not known chronic complications. Mean HbA1c before surgery was 7.55 ± 1.18 and 5.33 ± 0.59% at 2 years. Four months after the bypass only 12 patients maintained the diagnosis of diabetes. Two years after surgery the percentage of weight lost was 65.81% and the resolution of hypertension, dyslipidemia and diabetes occurred in 76.67, 89.29, and 92.68% cases respectively ($P<0.001$). According to the Framingham Risk Score, estimated 10 years cardiovascular risk was greater than 20% in 35.9% of cases before surgery. Mean risk decreased from 15.84% at baseline to 4.32% two years after surgery ($P<0.001$). 14.6% of patients had early complications and 19.5% developed later complications. BAROS scale was excellent in 36.8% of cases, very good in 36.7% and good in 21.1% at 2 years.
Conclusions
In morbid obese patients with type 2 diabetes, gastric bypass was shown as an effective tool in the early resolution of diabetes, besides its beneficial effects on 10 years estimated cardiovascular risk and quality of life.

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P765

Evaluation of the prevalence of obesity and overweight in the Belarus within the Framework of the National Campaign 'Early Detection and Prevention of Type 2 Diabetes Mellitus'

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The rapidly increasing epidemic of obesity is one of the most challenging dilemmas facing endocrinologists all over the world today. The aim of our research was within the framework of the National Campaign 'Early Detection and Prevention of Type 2 Diabetes Mellitus' to evaluate the prevalence of obesity and overweight in the Belarusian population.
Materials and methods
The Campaign involved opportunistic screening of individuals applying to the healthcare institutions of Belarus with the use of the FINDRISK (FINnish Diabetes Risk Score) scale. The evaluation of overweight was estimated on the basis of BMI and abdominal fat mass was assessed by waist circumference. Distriution by age and sex were taking into account.
Results
15 478 individuals participated in the Campaign. According to the survey data, 4977 (32.16%) respondents were aged under 45; 3926 (25.62%) respondents were aged between 45 and 54. These results demonstrate that almost 60% of the participants were of working age, an important fact. Gender breakdown of participants revealed that the majority of respondents were female 10 874 (70.25%) vs male 4604 (29.75%).
71.07% (10 875) of examined belorusian citizens had an excess body weight. Among them 44.09% (6824 respondents) were overweight; 26.17% (4051 respondents) were obese. Normal and BMI was registered in 28.93% (4603 citizens). The evaluation of waist revealed that 68% females had waist circumference above 80 cm and 54% males – above 94 cm, vs declared 32% females and 46% males with normal ranges. Abdominal adiposity as a predictor of CVD risk and the risk of

developing type 2 diabetes was registered in 934 (20.29%) males and in 3551 (32.66%) females.
Conclusions
The results of the National Campaign 'Early Detection and Prevention of Type 2 Diabetes Mellitus' testify to the presence of high percent of overweight (44.09%) and obesity (26.17%) in Belarusian population.

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P766

No long-term weight reduction after gastric banding (LAGB) in obese patients with craniopharyngioma involving hypothalamic structures: experiences from KRANIOPHARYNGEOM 2000

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Background
Craniopharyngiomas are embryogenic malformations which lead to eating disorders and morbid obesity due to hypothalamic involvement. The experience with laparoscopic adjustable gastric banding (LAGB) in obese craniopharyngioma patients is limited especially in regard to long-term effects and tolerability.
Patients and methods
We are reporting on four patients with childhood craniopharyngioma diagnosed at age 2, 13, 12, and 20 years.
Results
BMI–SDS at diagnosis was $-0.9, +4.5, +4.7$ and $+0.23$ S.D.. All patients developed morbid obesity (BMI–SDS: $+10.87, +10.36, +11.4, +6.2$) so that 11, 5, 9 and 3 years after diagnosis LAGB were performed. LAGB were well tolerated. During long-term follow-up, the nadir BMI–SDS ($+6.9, +9.5, +7.8, +4.9$) were reached 2.0, 0.5, 1.0, 0.8 years after LAGB. At last evaluation 9.1, 5.3, 7.1, 7.1 years after LAGB, the patients BMI (BMI–SDS at last evaluation: $+10.2, +13.9, +10.2, +6.3$) had increased again but remained at a constant level comparable with baseline BMI–SDS at the time of LABG. Quality of life was not decreased due to LAGB and tolerability was sufficient.
Conclusions
We conclude that LAGB is feasible and could have clinical relevant effects on long-term weight stabilization of obese craniopharyngioma patients with hypothalamic syndrome. However, a significant weight reduction was not achieved after LAGB in patients with childhood craniopharyngioma. Non-reversible bariatric procedures such as gastric bypass are not recommended for treatment of obese children and adolescents with craniopharyngioma due to ethical considerations.

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P767

Long-term weight development and psychosocial status in childhood craniopharyngioma patients

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Background
Craniopharyngioma (CP) are the most common sellar tumors in children. Patients often develop excessive weight gain and obesity due to several factors as involvement or damage of the hypothalamus. Previous studies on the weight development in craniopharyngioma patients have shown an increase in weight before and in the first 10 years after diagnosis leading to an impaired quality of life. The long-term weight development in these patients has not been investigated till now.
Methods
In a retrospective study, we analysed the weight development of 108 craniopharyngioma patients who were diagnosed before 2001. Data from physical examinations, anthropometric measurements and the patient's records were used, as well as a questionnaire answered by the patients in 2011 on their current weight and psychosocial status. The BMI of CP patients at diagnosis, 8–12 years after diagnosis, during long-term follow-up and at the time they answered the questionnaire was analysed and factors were investigated for their effect on the weight development.
Results
Long-term survivors of CP were assessed at a median age of 26.1 years (range 14.8–42.7) after a median follow-up of 17.01 years (range 8.81–33.40) after CP diagnosis. All patients show an increase in BMI during the first 10 years after diagnosis, as previously published. However, during long-term follow-up (more than 12 years after diagnosis) no further weight increase is seen. Patients with hypothalamic involvement of CP develop a higher initial weight increase, but also a stabilisation of BMI as well. Patients with a normal BMI at diagnosis (-2 to $+2$ S.D.) show the highest weight increase during the first 10 years after diagnosis, whereas patients presenting with obesity at diagnosis (BMI >3 S.D.) show a smaller increase in BMI–SDS during long-term follow-up.
Conclusion
We conclude that the degree of obesity in CP reaches a certain plateau during long-term follow-up.

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P768

Assessment of dietary habits in obese patients

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Introduction
A key factor in the progressive increase in the prevalence of obesity is poor dietary habits. The treatment of obesity with regular diets often fail, so knowing the specific habits of these patients can be very useful to individualize treatment.
Objective
Evaluate the dietary habits of subjects consulting for obesity in our clinic of Endocrinology and Nutrition
Materials and methods
We conducted a survey of eating habits for overweight and obese patients (Brown *et al*., Castro *et al*.) in all patients who consulted for this reason during previous 2 months.We collected data on age, sex, cardiovascular risk factors, anthropometric (weight, height, BMI, waist circumference) and laboratory parameters.
Results
Data from 68 patients were collected. The mean age was 52.8 ± 17.5 years, 34.4% males. 64.7% had diabetes, with a mean HbA1c of 7.52%, 50% were hypertensive and 58.8% dyslipidemic. Mean BMI at first visit was 39.77 ± 5.45 and BMI at the time of the survey was 37.62 ± 7.24 (66.7% between 25 and 40% and 33.3% over 40%). Mean weight: $112.0 \pm$ 24.36 kg initially vs 105.1 ± 21.77 kg finally, with a mean follow-up of 2.5 ± 2.1 years. 81.3% had received dietary advice by a dietitian. 28.5% of patients responded correctly to more than 75% of questions, 39,% between 50 and 75% and 32.1% responded adequately to $<50\%$. Subjectively, patients thought their diet was poor, fair, good, very good and excellent in 3.6, 53.6, 32.1, 10.7 and 0% respectively. There was a significant association between subjective scores and dietary questionnaire, so that subjects who responded appropriately to more than 75% felt that their diet was poor, fair, good or very good in 0, 25, 50 and 25% of cases and subjects who responded correctly to $<50\%$ of questionnaire felt that their diet was poor in 11.1%, fair in 77.8% and good in 11.1% (P 0.007). Presence of adequate habits (over 75% correctly responses) was significantly associated with instruction by the dietitian (34.8% in instructed vs 0% in subjects not instructed, P 0.047) but there was no association to any other features studied: BMI (22.2% in morbid vs 23.5% in no morbid obese), sex (35.3% in women vs 18.2% in men), age (31.6% in >40 years vs 22.2% in <40 years), diabetes (25% in diabetics vs 33.3% in non-diabetics), hypertension (35.7 vs 21.4%) or dyslipidemia (28.6 vs 28.6%)
Conclusions
Although dietary education improves eating habits of obese patients, a high percentage remain with inadequate dietetic costums, which could be a key factor in the failure of obesity treatment.

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P769

Sub clinical hypercortisism in patients with metabolic syndrome

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Objectives
Patients with metabolic syndrome who presents with vascular, metabolic or bone disorders of recent onset, or are difficult to control, or are progressively

deteriorating is potentially linked to cortisol excess the aim of the study is to evaluate the prevalence of occult CS in metabolic syndrome patients.
Method
In a cross-sectional study, 30 females with metabolic syndrome chosen from Endocrinology Clinic without any phenotypical features of Cushing's syndrome were subjected to: full history taking,clinical evaluation, BMI, waist circumference, fasting and pp blood sugar, lipid profile and overnight 1 mg dexamethasone suppression test (DST).
Results
Among the 30 patients in the study, three patients (10%) didn't show positive dexamethasone suppression test (occult Cushing's syndrome) and the rest of the patients (27) showed positive test
Conclusion
A relatively high prevalence of occult CS was found in our study. Further studies are needed to provide a rationale for systematic screening of occult CS in this population. To be of benefit by reducing the period of time spent in the atherosclerotic, catabolic, and thrombotic milieu of hypercortisolism
Keywords
sub-clinical, Cushing's, diabetes, metabolic syndrome.
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P770

Testosterone concentrations in obesity, an outcome of lipotoxicity?

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Objective
Testosterone levels are decreased in male obesity, the mechanism of which is incompletely understood. The aim of this study was to explore determinants affecting the feedback mechanism of the hypothalamic–pituitary–gonadal axis in male obesity and type 2 diabetes (DM2), such as aromatase expression and lipotoxicity.
Materials and methods
Circulating levels of testosterone were quantified (<1000 h with LC–MS/MS) in 37 middle-aged morbidly obese men (of which 23 had DM2) and 19 non-obese controls of similar age. LH and triglyceride levels were measured through standardized assays and aromatase expression was determined in subcutaneous adipose tissue biopsies using real-time PCR analysis.
Results
Mean testosterone levels were different among the groups, with 572, 372 and 207 ng/dl in non-obese, morbidly obese and morbidly obese men with DM2 respectively ($P<0.01$). Statistical differences in LH levels could not be established. Median triglyceride levels were higher in obese men vs non-obese men ($P<0.05$), with 96, 154 and 148 mg/dl in non-obese, morbidly obese and morbidly obese men with DM2 respectively. In non-obese men, age-adjusted Pearson's analysis showed that testosterone levels correlated positively with LH ($r=0.490$, $P=0.054$) and inversely with aromatase expression ($r=-0.635$, $P=0.036$). In obese men, an inverse association was found between testosterone and triglyceride levels ($r=-0.715$, $P=0.013$). Although in obese men with DM2 correlations with testosterone could not be established, LH was inversely correlated with triglyceride concentrations ($r=-0.535$, $P=0.010$).
Conclusion
Although testosterone in normal men may depend on aromatase activity, these data show that testosterone concentrations in male obesity are inversely associated with triglyceride concentrations, which have been suggested as a marker of lipotoxicity.
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P771

Hypogonadal men receiving testosterone treatment for 5 years had significant and clinically meaningful reductions in weight and waist circumference

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Objective
This study investigated effects of testosterone replacement therapy (TRT) on the metabolic syndrome in hypogonadal men over 5 years.
Methods
Cumulative, prospective registry study of 164 men with erectile dysfunction and testosterone below 12 nmol/l presenting to a urological institution. All patients received testosterone undecanoate injections 1000 mg every 12 weeks following an initial 6-week interval for a total of 5 years.
Results
After 5 years, mean weight decreased from 104.35 ± 13.84 (minimum 74.0, maximum 141.00) to 92.49 ± 11.15 (minimum 67.0; maximum 124.0). This decrease was statistically significant vs baseline ($P<0.0001$) and each year compared to the previous year. Mean change from baseline was $-9.79\pm0.35\%$. After 5 years, 96% of men had lost any weight, 82% had lost $\geq5\%$, 58% $\geq10\%$, 34% $\geq15\%$, and 18% $\geq20\%$.
Waist circumference (cm) decreased from 108.61 ± 10.13 (minimum 88.00; maximum 148.00) to 99.03 ± 9.05 (minimum 85.00; maximum 137.00). 98% had any reduction in waist size, 84% ≥5, 48% ≥10, 15% ≥15 cm. BMI declined from 33.21 ± 4.23 (minimum 24.96; maximum 43.10) to 29.42 ± 3.37 (minimum 19.58; maximum 37.39) ($P<0.0001$ vs baseline for all values).
Fasting glucose decreased from 112.77 ± 35.87 to 99.02 ± 18.1 mg/dl, HbA1c from 6.73 ± 1.27 to $5.63\pm0.64\%$. Total cholesterol declined from 264.24 ± 49.16 to 212.2 ± 40.88 mg/dl, LDL from 162.31 ± 26.28 to 126.41 ± 33.7 mg/dl, triglycerides from 261.07 ± 89.16 to 199.04 ± 52.34 mg/dl. HDL increased from 40.15 ± 13.04 to 55.62 ± 14.66 mg/dl. Systolic blood pressure decreased from 140.01 ± 13.61 to 122.35 ± 5.96 mmHg, diastolic from 81.67 ± 9.55 to 77.68 ± 4.07 mmHg ($P<0.0001$ for all).
Conclusions
TRT resulted in improvements of all components of the metabolic syndrome. Reductions of weight and waist circumference were progressive and statistically significant over the full 5 years of the study.
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P772

Moderating role of cognitive ability on the obesity: inflammation: insulin resistance triplet

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Obesity, the condition resulting from excess of body fat, is often accompanied by chronic low-grade levels of inflammation. These have been strongly associated with the development of insulin resistance (IR). Elevated BMI has also been linked to reduced cognitive performance, especially in executive functions, independently of medical co-morbidities.
Aim of this research is to investigate whether higher cognitive resources may present a moderating, 'protective' role on the triplet obesity – inflammation – insulin resistance.
Community-dwelling adults ($n=127$, M age $=37.35$, S.D. $=14.17$, range 17–65 years; 85% females) free of major and chronic autoimmune or inflammatory diseases underwent blood test after overnight fasting. C-reactive protein (CRP), a major marker of systemic inflammation, and Homeostatic Model Assessment, a widely used method to yield IR from fasting plasma insulin and glucose concentrations, were measured. Cognitive resources were evaluated through the General Adult Mental Ability Scale (GAMA), a non-verbal measure of general intelligence.
Moderated mediation analyses were performed comparing two alternative models. In model 1 a conditional indirect effect of BMI (obesity) on HOMA (insulin resistance), which is mediated by CRP (inflammation) was tested. Our interim results showed that the strength of the association between BMI and CRP varied at different levels of cognitive ability: the effect of obesity on inflammation was stronger for persons with poorer cognitive ability (controlling for age and education level). In a second proposed model, a direct effect of CRP (inflammation) on HOMA (insulin resistance), moderated by GAMA (cognitive ability) was tested. BMI was found to exert a direct effect on HOMA but the association between CRP and HOMA was not affected by cognitive ability.
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P773

Should all the obese patients be screened for Cushing's syndrome?

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Background
Cushing's syndrome (CS) is a relatively unusual condition that resembles many of the phenotypic features of obesity. The 1-mg dexamethasone suppression test (1 mg-DST) is the most frequently used screening tool for CS. However, the lack of suppression after 1-mg DST may be found in obese patients. In the current study, our aim was to evaluate the clinical signs of CS and the 1 mg-DST in obese patients.
Materials and methods
354 patients (87.9% females, age 37.8±13.4 years) who admitted to our outpatient clinic for obesity enrolled in this study. All the patients were evaluated for the clinical signs of CS. Weight, BMI, waist circumference, blood pressure were recorded. None of the patients used pharmacological glucocorticoid therapy. Lipid parameters, fasting glucose (FPG) and insulin, 75 g OGTT, basal cortisol and ACTH were measured. 1 mg-DST was performed.
Results
The mean weight of the patients was 102.4±20.1 kg, BMI 40±7.35 kg/m^2 and waist circumference 114.62±14.15 cm. 34.5% of the patients were hypertensive, 46.5% had prediabetes and 12.0.% had type 2 diabetes, 72.6% had dyslipidemia. 36.2% of the patients had central obesity, 72% dorsocervical fat accumulation, 28.8% abdominal striae and 23.2% acne. 49.4% of the women had hirsutism. The mean FPG, insulin levels and HOMA-IR were 112.49±46.59 mg/dl, 12.01±7.16 μU/ml and 3.11±2.03 respectively. The mean cortisol and ACTH levels were as follows: 9.28±3.53 μg/dl and 17.02±10.43 pg/ml. Seven patients failed to suppress plasma cortisol to <1.8 μg/dl. Biochemical confirmation tests were performed in these patients and two of them were diagnosed glucocorticoid-secreting adrenal adenoma.
Conclusions
As a result of our study, 0.5% of the obese patients were diagnosed Cushing's syndrome and 1.4% of the patients had false-positive 1 mg-DST.

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Diabetes remission after bariatric surgery may be jeopardized by remission criteria and previous hypoglycemic treatment

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Introduction
Controversy exists regarding type 2 diabetes (T2D) remission rates after bariatric surgery (BS) due to heterogeneity in its definition and patients' baseline features. We evaluate T2D remission using recent criteria, according to preoperative characteristics and insulin use.
Materials and methods
Retrospective study from a cohort of 657 BS performed in a single centre (2006–2011) of which 141 (57.4% women) had T2D. Evaluation of anthropometric and glucose metabolism parameters before surgery and at 1-year follow-up. Definition of T2D remission according to Buse et al: HbA1c <6%, fasting glucose (FG) <100 mg/dl, absence of pharmacologic treatment. Analysis of diabetes remission according to previous treatment.
Results
Preoperatively (mean ± s.d.): age 53.9±9.8 years, BMI 43.7±5.6 kg/m^2, T2D duration 7.4±7.6 years, FG 160.0±54.6 mg/dl, HbA1c 7.6±1.6%. 56 (39.7%) individuals had insulin therapy (IT). At 1-year follow-up, 74 (52.5%) patients had diabetes remission. %WL, %EWL and preoperative C-peptide levels were associated to remission (35.5±8.1 vs 30.2±9.5%, P=0,001; 73.6±18.4 vs 66.3±22.8%, P=0.037, 4.9±2.1 vs 3.0±1.8 ng/ml, P=0.001 respectively). Duration of diabetes, age and female sex were associated to non-remission: 10.3±9.4 vs 4.7±3.8 years, P=0.000; 55.1±9.3 vs 51.2±9.9 years, P=0.017; 58.9 vs 33.3%, P=0.004 respectively. Prior treatment revealed differences in remission rates: 67.1% in case of oral therapy (OT) vs 30.4% in IT, P=0.000. After adjusting remission rates to OT/IT, differences in duration of diabetes persisted.
Conclusions
Buse criteria reveal lower T2D remission rates after BS than previously reported. Prior insulin use is a main setback for remission. Longer diabetes duration, lower %WL, %EWL and baseline C-peptide, higher age and female sex were associated to non-remission.

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P775

Should remission of type 2 diabetes mellitus be the foremost goal after bariatric surgery?

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Introduction
Remission of type 2 diabetes (T2D) is a yearned outcome after bariatric surgery (BS). Attention to individuals who do not strictly fulfil remission criteria has been frequently left behind. The aim of this study was to evaluate metabolic control status in patients considered as diabetes 'non-remitters'.
Materials and methods
Retrospective study of 125 patients (59.2% women) with preoperative diagnosis of T2D who underwent BS in a single centre (2006–2011). Anthropometric and metabolic parameters, before surgery and at one-year follow-up. Definition of T2D remission according to Buse *et al*.: HbA1c <6%, fasting glucose (FG) <100 mg/dl, absence of pharmacologic treatment. Analysis of non-remitters and their metabolic status according to ADA's target recommendations of glucose and lipid control: HbA1c <7%, c-LDL <100 mg/dl, triglycerides <150 mg/d, cHDL >40 (male) or >50 mg/dl (female). Statistics: ANOVA.
Results
Preoperatively (mean±s.d.): age 53.5±9.7 years, BMI 43.5±5.6 kg/m^2, duration of T2D 7.7±7.9 years, FG 162.0±56.3 mg/dl, HbA1c 7.7±1.6%. At baseline, ADA's target recommendations were present in 12 (9.6%) and at one year follow-up in 45 (36%) individuals (P=0.000). 62 (49.6%) patients did not achieve diabetes remission; 26 (41.9%) had now diet treatment only, 30 (48.4%) oral medications and 6 (9.7%) required insulin. Of the non-remitters, 57 (91.9%) had HbA1c <7 and 18 (40.0%) achieved ADA's target recommendations. There were no differences between remitters and non-remitters in the number of individuals reaching ADA's target glucose and lipid levels.
Conclusions
Although almost 50% of patients may not be classified as diabetes remitters, they achieve a significant improvement in glucose and lipid control, which should be considered a success according to most scientific societies' target recommendations.

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P776

Development of metabolic syndrome is influenced by the thyroid function and age

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A lot of new data have been recently generated regarding the complex relationship between the thyroid function and metabolic parameters.
The aim of the study was to estimate the obesity and metabolic syndrome (MS) rates according to the age and the thyroid function in the residents of Belarus.
We have examined 894 subjects aged 18–44 years (82.2% females) living in Stolin district, Brest region of Belarus. The endocrinological examination with anthropometric measurements, the thyroid ultrasound, thyroid function tests and blood biochemistry were performed. The central obesity was diagnosed according to the IDF criteria.
Waist circumference exceeded the IDF cut-off levels in 68.1% of the studied population (71.8% females and 50.9% males). The MS (central obesity +2 or more the IDF factors) was diagnosed in 29% of the whole population: only in 5% of subjects at the age of 18–19, in 19% of 20–29 years old, in 32% of aged 30–39

years and in 48% of those aged 40–44 years. In the population of Stolin, 87% of the studied people were euthyroid (TSH 0.3–4.0 mIU/l). Hyperthyroidism (TSH <0.3 mIU/l) and hypothyroidism (TSH >4.0 mIU/l) was found in 2 and 11% of subjects respectively. The frequency of MS in subclinical hypothyroidism was significantly higher than in the group with normal thyroid function (39 vs 27%, $P<0.001$). In young participants (20–29 years old), the proportion of those having MS was twice higher in subclinical hypothyroidism compared to euthyroidism (33 vs 15% respectively). Such a tendency was decreasing with aging.

Our screening study has demonstrated the most evident influence of hypothyroidism on MS development in young people (20–29 years old). Such an effect disappeared in the older age groups, even if the rate of MS was higher in them.

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P777

Frequency of attention-deficit/hyperactivity disorder (ADHD) in a bariatric post surgery sample: clinical, analytical and psychological differences among bariatric patients with ADHD criteria

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Background

There is an increasingly aware of strong associations between obesity and attention-deficit/hyperactivity disorder (ADHD), with high rates of ADHD (26–61%) in patients seeking weight loss. Psychological evaluation is mandatory in patients presenting for bariatric surgery (BS), although it is focused on eating disorders and depression, with data lacking on ADHD. ADHD could increase the risk for less successful results after surgery because of noncompliance with recommendations.

Aims

To determine the frequency of ADHD in a BS sample. To investigate if there are any differences among clinical, analytical and psychological parameters in individuals with ADHD.

Methods

Sixty patients (78.3% females, age 46.35 ± 9.89, months since BS 46.28 ± 28.1) who underwent BS, with a minimum follow up of 12 months, were evaluated cross-sectionally. Initial and current BMI, eating patterns, comorbidity, socio-demographic and biochemical parameters were recorded. For the screening of ADHD, ASRS-v1.1 was administered.

Results

Nineteen individuals out of sixty (31.6%) had a positive screening for ADHD. This group had higher levels of HDL-cholesterol (62.84 ± 17.35 vs 53.59 ± 9.92 mg/dl; $P=0.011$) and Apo-A (177.79 ± 28.48 vs 154.94 ± 34.77 mg/dl; $P=0.015$), as well as an increased consumption of lipids (42.2 ± 7.12 vs $36.76\pm8.34\%$; $P=0.019$). The average intake time per meal was lower (13.89 ± 9.99 vs 20.49 ± 11.55 min; $P=0.036$). Also, there was a greater personal history of prebariatric psychiatric disorders (84.2 vs 48.8%: $P=0.009$), being the commonest depression ($n=12$) and anxiety disorder ($n=5$). Furthermore, there was a tendency of developing eating disorders (52.6 vs 21.9%; $P=0.059$). ADHD subjects had more difficulties in following visits after BS (52.6 vs 24.3%; $P=0.011$). We could not find any differences in achieved BMI, depressive symptoms or quality of life.

Conclusions

Patients of BS with ADHD criteria face significant difficulties with compliance in follow-up, but we could not find differences in major clinical outcomes. Surprisingly, this group of patients could have a protective lipid profile, probably related to dietary habits.

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P778

Do the association of phthalates and some parameters of obesity persist? Pilot study

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Background

Phthalates present endocrine disruptors widely used as plasticisers, solvents and additives in many consumer products. Experimental data and human cross-sectional studies suggest association of phthalates and obesity. This study focused to association between urinary levels of some phthalate metabolites and BMI and waist circumference.

Methods

We recruited 87 obese persons (44 females and 43 males), age 38.57 ± 8.14 years and 103 healthy controls (52 females and 51 males) similar age. Waist circumference was measured and BMI was calculated. Phthalate monoester metabolites (monoethylphthalate (MEP), monobuthylphthalate (MBP), and mono-(2-ethylhexyl) phthalate (MEHP)) were measured in single spot urine by mass spectrometry.

Results

BMI (35.28 ± 8.14 vs 22.85 ± 2.10 kg/m^2, $P<0.001$) and waist circumference (110.85 ± 15.35 vs 78.38 ± 8.03 cm, $P<0.001$) were significantly higher in obese than in controls. All urinary phthalate metabolites were higher in obese than in controls, but non significant (MEP 52.53 ± 141.89 vs 32.89 ± 128.99; MBP 21.52 ± 134.30 vs 10.25 ± 79.44; MEHP 20.83 ± 56.40 vs 19.90 ± 56.94 ng/ml). There are no any differences in levels urinary phthalate levels between genders in both groups. Only MBP had significant positive correlation with BMI ($r=0.201$; $P<0.05$) and waist circumference ($r=0.198$; $P<0.05$) in all participants who were positive for urinary phthalate metabolites.

Conclusion

The association between dibutylphthalate (DBP) and some parameters of obesity is possible. Further studies have to confirm and elucidate this association.

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P779

'Diabesity' in women: the EEM study

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'Diabesity' is the term for diabetes occurring in the context of obesity. Obesity is a cardiovascular major risk factor. The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) report designated diabetes as a coronary heart disease equivalent. Cardiovascular disease is the leading cause of death in women. The 'Estudio Epidemiologico de la Mujer' (EEM) study aimed to explore obesity, diabetes and diabesity in 3500 women over 18 years old. EEM is a cross-sectional epidemiological and multidisciplinary study in seventy consulting rooms of gynecology from twenty two Venezuelan provinces. Hypertension, hypercholesterolemia and metabolic syndrome defined according to NCEP-ATPIII, were surveyed among other cardiovascular risk factors. Cardiologists, gynecologists and endocrinologists participated as investigators in this study. We present the results of 10% of the sample collected to date. Mean (S.D.) age of the participants was 42.25 (13.41) years The prevalence of Obesity and Diabetes was 23.55% (95% CI: 19.64–27.45) and 3.94% (95% CI: 1.92–5.97) respectively. Diabetes was present in 7.14% of obese women. Obesity was detected in 42.86% of diabetic women. Patients with 'Diabesity' had higher prevalence of hypertension (83.3%, P 0.017), hypercholesterolemia (66.7%, P: 0.032) and metabolic syndrome (83.3%, P: 0.000). The prevalence of 'Diabesity' and its components was substantial across Venezuelan women. EEM study findings, including evidence of the association of 'Diabesity' with hypertension, hypercholesterolemia and metabolic syndrome, should inform appropriate clinical and public health interventions.

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P780

The medical and moral obligation of healthcare professionals to address complications in obesity during pregnancy

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Summary
This audit highlights a shortfall in healthcare provision when discussing the potentially sensitive issue of obesity. Given the significance of complications in pregnancy, and in future life, discussing such issues openly and encouraging patient responsibility without blame is integral to optimise antenatal benefit.
Introduction
The prevalence of obesity is increasing in the UK. Obesity in pregnancy is associated with various maternal and fetal complications. In the latest CMACH (2011) report, 30% of maternal deaths were associated with obesity. Appropriate antenatal interactions between practitioners and patients are crucial to ensure behaviours are adopted to minimise these risks.
Materials and methodology
An audit was performed comparing practice against CNST Criterion 10: obesity. Thirty sets of notes were randomly selected; ten from each BMI class.
Results
All of the patients had BMI's recorded in the notes and were booked for appropriate maternity based care. However, only 3 out of 30 patients (10%) were explained the antenatal and intrapartum risks associated with obesity in pregnancy.
Discussion
A complex interaction exists between the appropriate balance between medical obligations and moral issues when communicating with obese patients. The media portrayal of obesity as a moral failure has resulted in discrimination towards such patients and consequent difficulty in engaging in sensitive conversations about potentially serious complications. Honest and open consultations, addressing responsibility and control, but without blame, are essential to invoke behavioural change. Adopting such behaviours antenatally is essential to minimise the significant risks associated with obesity in pregnancy.

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P781

Circulating adiponectin as marker of obesity-induced chronic low grade inflammation

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Introduction
Obesity lowers adiponectin levels. Adiponectin has anti-inflammatory effects. It has been suggested that the lower levels of adiponectin in obesity results in chronic low grade inflammation (CLGI). Aim of this study was to examine if plasma adiponectin is a valid marker of CLGI in normal weight, overweight and obese individuals. In addition, we also assessed adiponectin as marker of body fat and insulin resistance
Methods
We measured adiponectin, markers of CLGI (CRP, SAA, ESR), insulin resistance and lipids. Body fat was estimated by DEXA and BIA.
Results
i) Adiponectin was widely distributed within each BMI group. ii) The levels of makers of inflammatory and insulin resistance were progressively and significantly elevated in parallel to the increased BMI. iii) In our combined population, adiponectin was significantly and negatively correlated to BMI, lipids and body fat while no correlation was evident with markers of CLGI. iv) After adjusting for BMI, a positive and moderate correlation between adiponectin and body fat was evident only in the obese. v) The distribution of adiponectin within each BMI group was wide and overlapping the three BMI groups its median value being 10.9 μg/ml. (vi) This phenomenon was not due to higher concentrations of adiponectin in the females since it was apparent in the males. (vii) Adiponectin levels in each BMI group were separated into quartiles which revealed: a significant reverse association of adiponectin with total and leg fat mass, a weak correlation between adiponectin levels and markers of CLGI, and a significant correlation to insulin resistance.
In conclusion
adiponectin is widely distributed within each BMI group. It appears to be a good marker of adiposity and insulin resistance but not a valid marker of obesity-induced chronic inflammation.

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P782

Prevalence of obesity, diabetes and prediabetes in Tirana, the capital of Albania 2010–2011

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Introduction
Obesity and diabetes are strongly associated as causes of morbi-mortality in developing countries. The aim of our study was to evaluate the prevalence of overweight, obesity and undiagnosed diabetes in Tirana district, where 1/4 of the total population lives.
Method
Anthropometric and capillary glucose measures for healthy people unknown of diabetes. Diabetes familiar anamnesis, HTA treatment and smoking were included in the questionnaire.
Results
3316 persons included in the study. M/F 1755/1561 (52.9%), mean age 53.4 ± 12.02 years, mean BMI 28.3 ± 1.9 kg/m^2. Prevalence of Obesity was 23.2% equally present M/F 23.4/23.1%, overweight 46.5%, more present in men 49.4/41.2% ($P<0.05$). Obesity was more frequent in group 40–60 years old – 27.25%, but 15.9% for the group 30–40 years old. Central obesity was present in 67.7% of cases, more frequent in women 79.9 vs 50.1% ($P<0.01$). Prevalence of undiagnosed Diabetes was **3.5%**, IFG 14.5**%**, IGT 10.6**%**. Diabetes was more frequent in the groups 20–30 years (5.41%) and 50–60 years 4.32%. The patient diagnosed with Diabetes had the tendency to be more overweight 53.3 vs 44.2%, to have more familiar anamnesis for diabetes ($P<0.01$) and suffer from HTA ($P<0.05$), but not obese 20 vs 21.5%. IFG and IGT were more frequent in the group 50–60 years old.
Conclusions
Prevalence of obesity, diabetes, and prediabetes is very high in Albania, especially in younger age group. It is important to raise the awareness and counseling of younger population about healthy living habits and weight control, as well as the frequency of controlling blood glucose level.

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P783

Madelung's disease: a case report

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Introduction:
Madelung's disease is a rare disorder characterized by a disturbance in the adipocyte metabolism. Mainly occurs in alcoholic Mediterranean male, presenting multiple, symmetrical lipomas.
Case report:
A 49-year-old obese man with a history of alcoholism, complaint of weight gain, soft and slow-growing swelling of the neck, and shoulders. His past history: epilepsy, alcoholic CHD, hypothyroidism, severe sleep apnea, vitiligo and HTA. His (BMI 52 kg/m^2), symmetrical subcutaneous masses between 10–18 cm, of nuchal, cervical, mandibular, upper back torso and upper and lower arms, freely movable, painless and non tender. Lab tests: IGT, and hepatic dysfunction. An abdominal CT scan: hepatic cirrhosis with intense proliferation of mesenteric fat. The diagnosis was made on the basis of history and clinical examination. Owing to portal hypertension, he is not eligible for gastric bypass surgery, and was enrolled in a nutritional plan.
Conclusion
This patient bears the hallmarks of this disease that, although rare, should not be underestimated. Abstinence from alcohol may only prevent further progression in the size of fat masses. Lipectomy and liposuction are the treatments of choice.

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P784

PTH one year after gastric bypass in morbidly obese patients

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Introduction
After gastric bypass, gastric secretion decreased and resorption of minerals and vitamins may be compromised. Lower resorpion of serum calcium and vitamin D could stimulate PTH secretion after restictive and malabsorptive bariatric procedures.
Methods
We have measured serum calcium, D vitamin and PTH in patients ($n=50$, 17 males and 33 females) 6 and 12 months after R en Y gastric bypass, 38.6 ± 12.6 years of age, with BMI 43 ± 9.2 kg/m^2. Exclusion criteria were thyroid dysfunction and previous primary or secondary osteoporosis. All patients were supplemented with calcium and vitamin D after bariatric surgery under the recommendations from European guideline for bariatric surgery. The supplements were contained 1250 mg of calcium carbonate and 400 IU of vitamin D in the first 2 months after surgery, and 600 mg of calcium citrate and 200 IU of vitamin D from the 3rd month to 1 year after gastric bypass.
Results
Serum calcium was 2.39 ± 0.93 mmol/l after 6 months and 2.41 ± 0.10 mmol/l after 12 months. The difference of -0.02 mmol/l was no significant ($P=0.83$). The serum level of vitamin D was 53.79 ± 24.60 ng/ml after 6 months and 46.73 ± 15.83 ng/ml after 12 months. The difference of 7.02 was no significant ($P=1.34$). After 6 months the mean PTH was 56.288 ± 25.429 pg/ml, after 12 months 46.646 ± 21.056 pg/ml, and that difference also was no significant ($P=0.94$).
Conclusion
One year after gastric bypass there was no significant changes in serum calcium, vitamin D and PTH level. Dietary supplements with calcium carbonate and D vitamin in the first 2 months, and with calcium citrate and vitamine D after that period in obese patients after restrictive and malapsorptive bariatric surgery procedures, is sufficiant supplementation model without increasing PTH and risk for bone resorption.

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P785

Neck circumference association with metabolic risk factors in patients undergoing neck surgery

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Adipose tissue distribution is one of the key determinants of metabolic dysfunction in obesity. Several large scale studies found that enlarged neck circumference is independently associated with cardiovascular risk.
The aim of this study was to examine the association of neck circumference with metabolic risk factors in patients undergoing neck surgery.
In 36 patients undergoing thyroid or vascular neck surgery we determined serum levels of insulin, glucose, triglycerides, HDL-cholesterol and C-reactive protein. We performed anthropometric measurements (body weight and height, BMI, waist circumference and neck circumference) and body composition analysis. We also evaluated carotid intima-media thickness and the presence of carotid plaques. Study participants did not have any inflammatory or malignant diseases. They also did not have any thyroid or other specific neck mass that could particularly enlarge neck circumference.
Statistically significant positive correlations were found between neck circumference, other anthropometric measures of obesity and body fat mass ($P<0.05$). Neck circumference also positively correlated with levels of insulin, glucose and triglycerides, but negatively with HDL-cholesterol ($P<0.05$). Neck circumference was associated with carotid intima-media thickness ($P<0.05$). Patients with carotid plaques had significantly larger neck circumference ($P<0.05$). In correlations with metabolic risk factors, neck circumference did not show any difference from waist circumference.
These preliminary results support the hypothesis that at least part of neck adipose tissue my be related to the metabolically adverse impact similar to ectopic fat. Samples of adipose tissue taken during neck surgery might bring further information about this particular fat depot. In that context we plan to proceed with investigations on neck adipose tissue samples from subcutaneous and perivascular locations.

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P786

Rapid reversal of hyperglycemia despite modest weight loss in a patient post gastric bypass surgery: a case report and review of the literature

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Background
Bariatric surgery has been shown to decrease insulin requirements even before significant weight loss has occurred. We report a patient with marked improvement in glycemic control despite modest weight loss post bariatric surgery.
Case presentation
A 47 years old Chinese man with obesity (weight 90 kg, BMI 31.2), hypertension, hyperlipidemia and type 2 diabetes mellitus diagnosed in 2005 is on follow up with our endocrine clinic. Despite compliance to more than 200 units of insulin a day, his glycemic control remained poor with a HbA1c persistently more than 10%. This is largely attributed to insulin resistance and dietary indiscretion. He underwent laparoscopic Roux-en-Y gastric bypass in mid 2012. Post-operatively, he experienced a total of 14 kg weight loss over 6 months. His HbA1c fell from 11.4 to 6.7% (within 4 months). All his diabetic and cholesterol lowering medications have been stopped.
Discussion
Resolution or remission of T2DM has been well documented following bariatric surgery in obese patients. This phenomenon is well reported after significant weight reduction. In this case study, the actual achieved weight loss was modest (15.6 vs 45.1% estimated weight loss in other demographically similar subjects), yet the glycemic control was comparable to similar subjects who lost much more weight in the same time period. This rapid reversal of hyperglycemia is not well understood and could partly be explained by improved beta cell function and insulin sensitivity following malabsorptive bariatric surgery. These outcomes have been linked to enhanced incretin responses along the gastroenteroinsular axis facilitating these improvements in glucoregulation.

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P787

Insulinresistance at obesity and diabetes

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The urgency of metabolic syndrome problem in modern medicine is caused by metabolic syndrome prevalence in the general population of 14–24%. The metabolic syndrome plays an essential role in cardiovascular diseases development acceleration. Components are abdominal–visceral obesity, insulinresistance, hyperinsulinemia, dyslipidemia, arterial hypertension and carbohydrate metabolism disorder.
Aim
Frequency estimate 2nd type diabetes and insulin resistance in obesity
Materials and methods
For metabolic syndrome severity and obesity degree verification the BMI was calculated. Immunoreactive insulin level was defined by the immunofermental analysis metod. The insulinresistance was defined by HOMA-index calculation.

Data are presented in a format of Me (Q25; Q75). The difference importance was estimated by Mann–Whitney's criterion.
Results
50 patients with obesity and without verified diagnosis of diabetes were examined. The age of the patients is 41.09 (27.88; 46.80) years. BMI is 42.88 (35.32; 46.24) kg/sq per m. The average immunoreactive insulin level of the patients is 17.70 (13.20; 23.70). At 50.0% of patients immunoreactive insulin level exceeded 17 μU/ml which is a hyperinsulinemia indicator. HOMA-index is 4.39 (2.87; 7.00). 70.0% of patients had the normal HOMA-index value excess that testifies insulin resistance formation.
During carried-out inspection it was established that 24% of patients had boundary glicemia value level from 6.00 to 6.40 mmol/l. 2nd Type diabetes is diagnosed for 8.0% of patients for the first time.
Output
This research confirms high insulin resistance prevalence and the asymptomatic diabetes course at patients with obesity.

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P788

Lean mass and fat mass distribution in Ukrainian women with metabolic syndrome in postmenopausal period

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Objective
Menopause-related changes in female body are associated with the greater risk of metabolic syndrome (MS), which includes obesity, dyslipidemia, impaired glucose tolerance, hypertension.
Aim
The purpose of our study was to reveal peculiarities of fat and lean mass distribution between postmenopausal women with abdominal obesity and with MS.
Design and Method
The sample consisted of 43 postmenopausal 60–69 years old women (age: mean = 64.8; s.d. = 0.4); duration of menopause: mean = 14.5; s.d. = 0.9). The diagnosis of MS was considered according to IDF (2005 year) criteria. Lean and fat mass distrubution were measured by dual-energy X-ray absortiometry, and were compared for the cohorts with and without MS. Data were analyzed using Statistical Package 6.0 (Statsoft).
Results
Findings revealed that 24 (55.8%) of postmenopausal women had MS. In patients with and without MS compared, fat mass was higher in the former group (41248.25 ± 2263.89 and 29817.68 ± 2397.78 respectively; $F = 11.9$; $P = 0.001$) and at different body regions also: gynoid fat (6563.72 ± 348.19 and 5115.21 ± 392.43 respectively; $F = 7.6$; $P = 0.008$), android fat (3815.45 ± 200.8128 and 2798.15 ± 282.79 respectively; $F = 9.06$; $P = 0.004$. Lean mass comparing didn't show significant differences in female with and without MS (42548.0 ± 1239.18 and 40667.53 ± 1223.78 respectively; $F = 1.1$; $P = 0.29$) and at different body regions also.
Conclusion
These findings suggest that in postmenopausal women with MS there is prevalence of fat mass without increasing of lean mass quantity in compare to female with abdominal obesity without MS.

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P789

Lean and fat mass in Ukrainian women of different age

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Aim
The aim of this study is evaluating of body composition and frequency of sarcopenia in women depending on age.
Materials and methods
We've examined 8637 women aged 20–89 years (mean age – 56.7 ± 0.14 years; mean height – 162.5 ± 0.07 cm; mean weight – 73.5 ± 0.16 kg). The patients were divided into two groups depending on age: 20–24 ($n = 143$), 25–29 ($n = 209$), 30–34 ($n = 271$), 35–39 ($n = 326$), 40–44 ($n = 419$), 45–49 ($n = 794$), 50–54 ($n = 1292$), 55–59 ($n = 1534$), 60–64 ($n = 1193$), 65–69 ($n = 943$), 70–74 ($n = 877$), 75–79 ($n = 384$), 80–84 ($n = 204$) and 85–89 years ($n = 48$). Lean and fat masses and total body, lumbar spine, femoral neck bone, forearm bone mineral density (BMD) were measured by DXA using a densitometer Prodigy, GE.
Results
We have found the significantly differences of fat and lean masses in women with age:
– fat mass: 20–24 years – 18 630.12 g; 25–29 years – 18 630.12 g; 30–34 years – 19 201.00 g; 35–39 years – 21 528.15 g; 40–44 years – 24 611.77 g; 45–49 years – 2750.54 g; 50–54 years – 27 501.54 g; 55–59 years – 29 909.92 g; 60–64 years – 31 600.27 g; 65–69 years – 33 508.25 g; 70–74 years – 33 155.54 g; 75–79 years – 32 284.86 g; 80–84 years – 30 595.53 g; 85–89 years – 30 303.68 g; $F = 83.19$; $P < 0.0000001$;
– lean mass: 20–24 years – 37 271.57 g; 25–29 years – 37 954.09 g; 30–34 years – 39 019.72 g; 35–39 years – 39 928.62 g; 40–44 years – 40 929.67 g; 45–49 years – 41 407.19 g; 50–54 years – 41 936.27 g; 55–59 years – 42 564.79 g; 60–64 years – 42 519.73 g; 65–69 years – 41 758.95 g; 70–74 years – 41 233.77 g; 75–79 years – 41 105.52 g; 80–84 years – 40 308.00 g; 85–89 years – 38 454.61 g; $F = 29.15$; $P < 0.0000001$.
Frequency of sarcopenia in women aged 65 years and older was 7% (women aged 65–69 years ($n = 943$) – 7.6% ($n = 72$), 70–74 years ($n = 877$) – 6.1% ($n = 54$), 75–79 years ($n = 384$) – 6.3% ($n = 24$), 80–84 years ($n = 204$) – 6.9% ($n = 14$), 85–59 years ($n = 48$) – 10.4% ($n = 5$).
Conclusion
Fat and lean masses were significantly decreased with age. The maximal accumulation of fat and lean masses was in women aged 50–59 years. Frequency of sarcopenia in women aged 65 years and older was 7%.

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P790

Dieting attitudes among college students in Romania

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Objectives
The aim of the study was to assess the eating attitudes and behaviors, including weight concerns and dieting behavior, among medical college students.
Methods
The sample consisted of 70 undergraduate students from the School of Medicine, Iasi, Romania, 22 males and 48 females, aged 25 ± 6 years. Eating-related behaviors were measured using the EAT–26 questionnaire. Study of food intake and physical activity was conducted using a questionnaire, that included anthropometric measures, food frequency and the level of physical activity. We calculated BMI from students 'self-reported height and weight.
Results
The mean BMI was 22.5 ± 4 kg/m^2, ranging from 16.2 to 31.7 kg/m^2. Approximately 13% of the students were overweight, and 7% were obese. Another 17% were underweight, and the remainder (63%) were of healthy weight. The results showed that 7% of the students had a positive EAT-26 score (> 20) and 10% scored higher on the dieting subscale. The results demonstrated a high prevalence of disturbed eating attitudes and behaviors among college students. Our analysis showed that students with dieting behavior report excessive exercising and consuming less cereals and meat and more legumes as compared to non-dieting behavior group.
Conclusion
The prevalence of eating disorders may be increasing in our country, because the young girls experiencing more cultural imperatives for thinness.

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Paediatric endocrinology

P791

GH treated children with IGF1 deficiency and excluded GH insensitivity despite normal GH secretion may attain similar final height as children with GH deficiency

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Introduction.
The diagnosis of GH deficiency (GHD) is based on decreased GH peak in stimulating tests (GHST). Recently, GHD has been re-defined as secondary IGF1 deficiency (IGFD). However, IGF1 may increase during GH therapy in the patients with normal GH peak in GHST, suggesting a diagnosis of non-primary IGFD (npIGFD).
The aim of the study was to compare GH therapy effectiveness in children with GHD and with npIGFD (responding to GH administration despite normal results of GHST).
Patients and methods
The analysis comprised 300 children (228 boys and 72 girls), with short stature and i)severe GHD (sGHD) – GH peak <5 ng/ml, height SDS at GH therapy onset (HoSDS) -3.20 ± 0.87 (mean $\pm$S.D.), $n=43$; ii) partial GHD (pGHD) – GH peak 5–10 ng/ml, HoSDS -3.06 ± 0.78, $n=188$; and iii) npIGFD – GH peak $>$ 10 ng/ml, decreased IGF1 (i.e. IGF1 SDS <-1.0), increasing significantly during generation test, HoSDS – 3.11 ± 0.70, $n=69$.
All the patients were treated with GH in a dose of 0.18 ± 0.03 mg/kg per week up to the attainment of final height (FH).
Selected auxological indices of GH therapy effectiveness were compared: i) FH SDS for age and sex; ii) FH SDS corrected by target height SDS (corrFH SDS); and iii) an increase of FH SDS with respect to HoSDS (ΔHSDS).
Results
The attained FH SDS was slightly worse in npIGFD (-1.48 ± 0.89) than in sGHD (-1.38 ± 1.25) but better than in pGHD (-1.62 ± 0.83), while corrFH SDS was similar in all the Groups (-0.32 ± 0.87 vs -0.38 ± 1.09 vs -0.39 ± 0.97 respectively). Moreover, ΔHSDS was similar in npIGFD (1.62 ± 0.88) and sGHD (1.68 ± 1.56), being even better than in pGHD (1.42 ± 0.95). All the differences among the groups were insignificant.
Conclusion
It seems that GH therapy should be considered in children with IGFD, responding to GH despite normal results of GHST, because the efficacy of treatment is similar as in GHD.

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P792

Reference curves for body fat (%) for Danish children evaluated by skinfolds and dual-energy X-ray absorptiometry

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Background
Over the last 60 years body composition of young people have changed towards increased fatness. Whole body fat percentage (%BF) derived from dual energy X-ray absorptiometry (DXA) scans are (although often not available) widely recognized as a better measure of fatness than BMI.
Objective
We aimed to provide reference materials for %BF of healthy Danish children evaluated by skinfold measurements and DXA and to compare sensitivity and specificity of different assessments of excess fatness.
Methods
Height, weight, and skinfolds, were measured in a large longitudinal cohort of Danish children from the Copenhagen area ($n=2647.12792$ examinations) between birth and 14 years. DXA scans were performed once at age 6–14 ($n=1200$). We calculated %BF from skinfold-measurements (Slaugther equation), evaluated number of overweight by BMI (Cole criteria) and evaluated increased fatness by DXA (using adult cut off values: males: 25%, females: 30%).
Results
Reference curves for %BF were constructed for boys and girls with generally higher values for girls and with broad intervals between $+1$S.D. and $+2$S.D. in both genders.
%BF from skinfolds were significantly lower (mean difference 13%) but correlated strongly ($r=0.88$) with DXA %BF, (and with similar Z-scores). BMI Z-score also correlated positively with DXA %BF ($r=0.74$), but a child with a normal BMI for gender and age ($-1<$BMI Z-score<1) could have a DXA %BF between 6 and 37. When applying adult cut off values for DXA %BF$\sim$1S.D. to identify children with excess fatness, only half of them were identified using BMI/Cole criteria (specificity of 99, but sensitivity of 50.5). Using %BF from skinfolds gave a specificity of 94.4 and sensitivity of 75.3.
Conclusions
Normal weight children may have too much fat. %BF derived from skinfolds measurements had a higher sensitivity than BMI when compared to the DXA when identifying children with excess fat.

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P793

Abnormalities in growth and in the IGF system can be associated to permanent chronic inflammatory process in HIV-infected children independently of clinical control

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Background
HIV-infected paediatric patients usually show impaired growth. Data reporting abnormalities in GH–IGF–IGFBPs system are scarce and inconclusive.
Aim
To analyse blood concentration of the major components of IGF–IGFBPs system in these children and compare them to growth parameters and to cytokines levels.
Methods
prepubertal HIV-infected children, aged 8.2 ± 1.7 years, were evaluated every 6 months during 1 year when anthropometric data and blood samples were collected for IGFs, IGFBPs, cytokines and viral load (VL) determinations. Thirty healthy prepurbetal children were studied as controls. IGF1, IGF2, IGFBP3 and IGFBP1 were determined by ELISA and IGFBP2 and IGFBP4 by western-ligand blotting (WLB). Interleukin 6 (IL6) and tumoral necrosis factor α (TNFα) were determined by Luminex. We defined VL <5.000 and VL >5.000 copies/ml as good (GC) and poor (PC) disease control respectively.
Results
BMI in HIV-infected children was similar to controls. Height was lower in HIV-children than in controls ($P<0.001$). Serum IGF1, IGF2 and IGFBP3 were similar in PC and in GC but lower than in controls. IGFBP1 and IGFBP4 levels were similar among PC, GC and controls. IGFBP2 levels were higher in PC than in GC. IL6 and TNFa concentrations were similar in PC and in GC but higher than in controls, indicating a permanent chronic inflammatory process in HIV-infected children. No significant correlation was observed between IL6 or TNFα and IGF1, IGF2 or IGFBP3. However, IGFBP1 levels were significantly lower (<58 ng/ml) in samples with TNFα >24 pg/ml ($P=0.0006$) or IL6 >3.8 pg/ml ($P=0.05$).
Conclusion
HIV-children present poor growth comparatively to healthy children. The poor growth may be explained by alterations in IGF-IGFBPs system that reduces IGF bioavailability/activity during good or poor disease control. The permanent and chronic inflammatory process may contribute to IGF–IGFBPs alterations.

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P794

Unaltered sex steroid levels, but elevated serum IGF1 in healthy boys with pubertal gynaecomastia

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Introduction
The presence of glandular breast tissue in males around puberty, pubertal gynaecomastia, is a very common (40–60%) condition although the aetiology behind is poorly understood. It is generally accepted that pubertal gynaecomastia is caused by an excess of estrogens and/or a deficit of androgens. However, other hormones such as prolactin, GH and IGF1 may also affect ductal growth of the breast.
Design
A cross-sectional study of 518 healthy Danish school boys (aged 6.1–19.8 years) as a part of the COPENHAGEN Puberty Study. Anthropometry and pubertal stages (PH1–6 and G1–5) were evaluated, and the presence of gynaecomastia was assessed. Body fat percentage was calculated by means of skin folds and impedance. Non-fasting blood samples were analysed for FSH, LH, testosterone, SHBG, estradiol, IGF1, IGFBP3 and prolactin. Furthermore free-testosterone, FAI and E_2/testosterone were calculated.
Results
We found that boys with gynaecomastia had significantly higher height, weight, hip circumference, testis volume, genital- and pubic hair stages, levels of FSH, LH, estradiol, testosterone and IGF1, but lower levels of SHBG. FSH ($P=0.029$) and IGF1 ($P=0.003$) remained significantly higher in boys with gynaecomastia even after adjustment for age and pubertal stage. We did not find any significant difference in the sex steroid levels or in estradiol/testosterone-ratio, FAI, or free testosterone.
Conclusion
We found that Danish boys with gynaecomastia had significantly higher serum IGF1 levels compared to boys without palpable gynaecomastia at the time of investigation. No other significant hormonal differences were seen. We suggest that the GH–IGF1 axis may be involved in the pathogenesis of pubertal gynaecomastia.

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P795

Metabolic syndrome in adolescents and young adults with childhood-onset GH deficiency

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Introduction
There is only few data on metabolic syndrome occurrence in young patients with childhood-onset GH deficiency (CO-GHD), especially its partial form.
Aim
The aim of this study was the assessment of the metabolic syndrome criteria (according to IDF 2007) occurrence in adolescents and young adults with CO-GHD and evaluation of their correlations with the degree of GH/IGF-1 axis function impairment.
Subjects and methods
The study was performed in a group of total 122 subjects aged 16–25 years. Based on current peak serum GH concentrations in insulin tolerance test (ITT) patients were qualified for one of the following groups: i) severe GH deficiency (GHD; peak GH <5.0 ng/ml, $n=26$), ii) partial GHD (PGHD; peak GH 5.0–10.0 ng/ml, $n=22$), iii) normal GH secretion (NGH; peak GH >10.0 ng/ml, $n=28$), iv) healthy subjects (H; $n=46$). Following examinations were performed: i) anthropometric measurements (body mass, height, hip and waist circumference), ii) blood pressure iii) serum glucose, insulin, total, HDL and LDL-cholesterol, triglycerides, IGF1, iv) HOMA-IR was calculated.
Results
WHR was significantly ($P<0.01$) higher in GHD (0.82) as well as in PGHD (0.78) in comparison with NGH (0.73) and H (0.73) subjects. We observed significantly elevated ($P<0.05$) total and LDL-cholesterol, triglycerides and hsCRP in GH, but not in PGHD compared to NGH or H groups. There were no differences in fasting glucose, insulin and HOMA-IR values between the examined subjects. Metabolic syndrome has been diagnosed in four NGH patients (15.4%). Significant ($P<0.05$) negative correlations between the peak ITT GH concentrations and hsCRP ($r=-0.51$), total ($r=-0.43$) and LDL-cholesterol ($r=-0.38$) and triglycerides ($r=-0.28$) were observed. Serum IGF1 correlated negatively with hsCRP ($r=-0.44$), glucose ($r=-0.43$) and HOMA-IR ($r=-0.31$) and positively with insulin ($r=0.29$).
Conclusion
i) Metabolic syndrome is significantly more frequent in adolescents and adults with GHD. ii) Partial CO-GHD is not associated with metabolic disturbances in our patients.

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P796

Premature pubarche: distinguishing between nonclassic congenital adrenal hyperplasia and idiopathic premature adrenarche

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Introduction
Premature pubarche (PP) is most often related to idiopathic premature adrenarche (IPA). However, it is a diagnosis of exclusion and differential diagnosis must include milder and nonclassic variants of congenital adrenal hyperplasia (CAH).
Purpose
To identify clinical predictors of CAH and IPA in children with PP.
Materials and methods
A retrospective study was conducted including children seen for PP between 2001 and 2011 with baseline blood sampling for DHEAS, androstenedione, 17OHP and free testosterone and a follow up of at least 1 year. Patients were considered to have CAH if their 17OHP level was ≥ 2.0 ng/ml and CAH was confirmed by mutational analysis of the CYP21 gene. Exclusion criteria were: neonatal onset, concomitant clinical signs of central puberty and genetic syndromes. Statistical analysis was done using SPSS 19th ($P<0.05$).
Results
Fifty-three children with PP were included: 6 had CAH (11.3%) and 47 were classified as IPA (88.7%). Pubic hair onset was reported by the parents at 4.9 ± 2.2 and 4.9 ± 1.6 years ($P=0.940$) and age at the first appointment was 7.1 ± 1.0 years and 6.9 ± 1.5 ($P=0.692$) in CAH and IPA respectively. There was no difference on stature SDS and BMI–SDS at the first appointment and after one year. Progression of pubic hair Tanner stage in the first year also didn't differ between the two groups. Growth velocity SDS was 1.34 ± 0.76 in CAH and 0.75 ± 1.06 in IPA ($P=0.195$). Bone age was advanced 1.9 ± 1.3 years in CAH and 1.1 ± 1.3 years in IPA ($P=0.175$). Besides 17OHP, also free testosterone was higher in the CAH group ($P=0.005$).
Conclusions
CAH cannot be distinguished from IPA on a clinical basis. Evaluation of androgens is essential to make the differential diagnosis in a child with PP.

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P797

Improvement in metabolic control of type 1 diabetes mellitus in a tertiary unit: 2005 vs 2012

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Aim
To define the main predictors of metabolic control in children and adolescents and evaluate its improvement along the years.
Methods
We included children and adolescents with 1DM, with more than two years of disease. Data were collected at 2005 and at 2012. Sex, age and severity at diagnosis, therapy in the last year, age at onset of multiple daily insulin injections (MDII) and continuous subcutaneous insulin infusion (CSII), number of group educational sessions, insulin daily dosis (IDD) and mean HbA1c along last year were collected. Three groups were defined to evaluate metabolic control (1, HbA1c $\leq 7.5\%$; 2, HbA1c 7.5–9%; 3, HbA1c $\geq 9\%$). Statistic analysis was performed with SPSS®.

Results
We included 243 childrens with 1DM (107 in 2005 and 136 in 2012). There were no differences in sex, age at diagnosis (6.0±3.3 vs 6.6±3.6 years) or duration of illness (6.8±3.3 vs 6.2±3.6 years). Severity at diagnosis was higher in 2005 ($P=0.021$). In 2005, 34.6% were in conventional therapy and 65.4% in MDII. In 2012, all children were in intensive therapy since diagnosis (75% MDII and 25% CSII). Comparing data from 2005 to 2012, we found statistical difference in number of group educational sessions (1.6±0.9 vs 4.8±2.5; $P<0.001$), duration of MDII (1.8±2.2 vs 3.5±1.1; $P<0.001$), IDD (1.04±0.27 vs 0.91±0.22 IU/kg/d; $P<0.001$), mean HbA1c in the last year (8.7±1.3% vs 7.7±1.0%; $P<0.001$), and groups of HbA1c (group1=17.8%, 2=52.3%, 3=29.9%; vs group1=47.1%, 2=45.6%, 3=7.4%; $P<0.001$). In 2012, the children in group 1 had started MDII at younger age ($P=0.04$) with lower IDD ($P=0.02$) and had more children in CSII ($P<0.001$); children in group 3 were older ($P=0.04$).
Conclusion
There was a clear improvement in metabolic control from 2005 to 2012. The main predictors of greater metabolic control were early onset of MDII and CSII and increase of educational sessions. This reinsures the advantage of intensive insulin therapy since diagnosis.

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P798

Subclinical hypothyroidism in obese children: the influence of L-thyroxin treatment on metabolic comorbidities and a success of dietary therapy

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Introduction
Subclinical hypothyroidism (sHT) is defined as elevated level of TSH with normal levels of thyroid hormone. In obese children there is a high frequency of this disturbance. However, the influence of sHT on therapy success and an appearance of metabolic complications in childhood obesity is unclear. Furthermore, the supplementation L-thyroxin (L-T_4) in this case seems to be very controversial.
Aim
The aim of this study was to evaluate of sHT appearance and L-T_4 treatment influence on metabolic complication and therapy success in obese children.
Materials and methods
Medical records of 55 obese children with sHT diagnosed underwent retrospective analysis. 29 children (group 1) L-T_4 treated with mean age 9.8 years and mean BMI – 27.1 kg/m^2. Not treated group: 26 children with mean age – 10.1 years.and BMI – 27.2 kg/m^2 (group 2). Both groups received dietetic and behavioural counselling. Anthropometrical parameters, metabolic complications and efficiency of dietary therapy were analysed in both groups.
Results
In 1 and 2 g respectively 86.2 and 80.8% of children showed up on a check-up visit. There was no significant difference in frequency of metabolic complications between both groups. Proportion of children that obtained the body mass loss was similar (48% in 1 g and 43% in 2 g). The efficiency of therapy presented by delta of BMI Z-score (S.D.) was also comparable (Δ Z-score BMI was respectively −0.55 and −0.63 S.D.). Normalisation of TSH was gained at 62% of children in group not treated with L-T_4.
Conclusions
sHT doesn't decrease the efficiency of dietary therapy in children. The L-T_4 treatment of sHT in children has no influence on frequency of metabolic complications and efficiency of therapy. The body mass reduction in obese children with sHT enables the normalization of TSH without the necessity of pharmacotherapy.

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P799

Urinary phthalates from 168 girls and boys measured twice a year during a 5-year period: associations with adrenal androgen levels and puberty

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Background
Little is known about the possible deleterious effects of phthalate exposure on endogenous sex steroid levels in children
Design
A longitudinal study.
Materials and methods
168 Healthy children (84 girls) were examined every 6 months for 5 years, with pubertal staging, measurements of serum levels of DHEAS and Δ4-androstenedione (Adione) and repetitive longitudinal morning urinary measurements of 14 phthalate metabolites, corresponding to seven different phthalate diesters.
A geometric mean (GM) excretion of each child was calculated as the GM urinary phthalate amount (ng) per kilogram (kg) body weight in consecutive urine samples.
Results
We found that girls with excretion of the sum of dibutyl phthalate isomers (MBP) and di(2-ethylhexyl) phthalate (DEHP) metabolites above geometric group mean (795 and 730 ng/kg respectively) had lower serum levels of DHEAS and Adione, although only statistical significant at 13 years of age. In boys we found that excretion of MBzP above geometric group mean (346 ng/kg) was associated with a lower serum level of DHEAS at 11 years of age. A lower age at pubarche was observed in the boys with 'high' excretion of the sum of dibutyl phthalate isomers (MBP) metabolites (11.0 vs 12.3 years).
Conclusion
We provided some evidence that excretions of the phthalates, MBP in girls and MBzP in boys, were negatively associated with adrenal androgen levels. However, the lower androgen levels were not correlated to changes in age at pubarche.
The lower androgen levels in girls in the 'high' excretion group of MBP did not appear to influence onset of puberty. However, the boys in the 'high' excretion groups of MBP and the molar sum of correlated phthalates appeared to start puberty earlier than the boys in the 'low' excretion group. Our study did not reveal any clues with regard to possible mechanisms, but we speculate that theoretically, phthalates may not just interfere with steroidogenesis but they could also act directly at the hypothalamic-pituitary level.

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P800

Hormonal and auxological data of the patients with persistent and transient GH deficiency, diagnosed according to different criteria after completion of growth-promoting therapy

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Introduction
In majority of patients with childhood-onset GH deficiency (GHD), normalisation of GH secretion at the attainment of final height (FH) is observed. However, different criteria for GHD diagnosis may be considered.
The aim of the study was to compare the auxological and hormonal data of the patients with persistent and transient GHD, diagnosed according to different criteria.
Patients and methods
The analysis comprised 150 patients (117 boys), age 12.5±2.7 years (mean±S.D.), with GHD, who completed GH therapy in a dose of 0.18±0.03 mg/kg per week, attained FH and were subjected to re-evaluation of GH secretion in stimulating tests (GH re-test) at the age of 17.3±1.1 years.
In the patients with confirmed and excluded GHD in GH re-test, using the following cut-off levels of GH peak: <3 ng/ml (persistent, severe GHD in adults), <6 ng/ml (GHD in young adults), <10 ng/ml (GHD in children), the following auxological and hormonal data before treatment and indices of GH therapy effectiveness were compared i) height SDS at therapy onset (HoSDS), ii) GH peak and IGF1 SDS before treatment, iii) FH SDS for age and sex, and iv) increase of FH SDS with respect to HoSDS (ΔHSDS).
Results
The patients with GH re-test <6 ng/ml had significantly ($P<0.05$) lower GH peak (3.9±3.2 ng/ml) and IGF1 SDS (−1.74±2.83) at therapy onset, together with significantly better FH SDS (−0.39±1.19) and ΔHSDS (2.98±2.14) than the patients with GH re-test >6 ng/ml (7.7±3.9 ng/ml, −1.26±1.71, −1.51±0.86 and 1.51±0.90 respectively), with no difference in HoSDS (−3.38±1.08 vs −3.05±0.73). There was no significant difference in GH therapy effectiveness in

the patients with GH re-test <3 and 3–6 ng/ml, as well as between ones with GH re-test 6–10 and >10 ng/ml.
Conclusion
The cut-off value 6 ng/ml for GH re-test seems to be the most appropriate for diagnosing persistent GHD in the patients who achieved FH.
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P801

Adipocyte dysfunction in pediatric obesity
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Background
Obesity is associated with adipocyte dysfunction, characterized by an impaired secretion of adipokines, which leads to a systemic inflammatory status.
Aim
To characterize adipokines' profile in a group of obese children and adolescents.
Method
A case–control study comparing 102 obese children (BMI $\geq$95th percentile; aged 10–18 years) to a group of 43 healthy controls matched for age and pubertal status.
Adiponectin, leptin, resistin, TNFα, AFABP and lipocalin-2 were measured using ELISA method and were correlated with traditional clinical and biochemical biomarkers of the metabolic syndrome (BMI, waist circumference, blood pressure, fasting glycaemia and insulinemia, and lipids profile).
Results
The plasmatic levels of leptin, resistin, AFABP, lipocalin-2, and IL6 were significantly higher while the adiponectin plasmatic levels were significantly lower in the obese group compared to the control group; TNFα was not different between groups.
Adiponectin, leptin, AFABP, and resistin were significantly correlated to BMI, blood pressure, and insulin resistance biomarkers (negative correlation for adiponectin, positive correlations for others adipokines); there was also a parallel variation of the previous mentioned adipokines and HDL-cholesterol, positive for adiponectin and negative for leptin, resistin, and AFABP.
IL6 and lipocalin-2 were positively correlated only to BMI, while TNFα was positively correlated to systolic blood pressure.
Conclusions
Pediatric obesity is already associated with an altered production of adipokines, which participates in the pathogenesis of obesity-associated comorbidities.
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P802

GnRH analog treatment in children with congenital adrenal hyperplasia complicated by central precocious puberty
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Introduction
In children with congenital adrenal hyperplasia (CAH), central precocious puberty (CPP) may occur and this situation may compromise final height. We aimed to evaluate the effect of GnRH analog therapy (GnRHa-T) on growth in children with CAH.
Design
Ten children with CAH were included in ongoing follow-up study. Nine children underwent GnRH stimulation test. GnRHa-T was used as 3.75 mg/q 4 weeks and the dose had to be increased to 7.5 mg/q 4 weeks in two children. Bone ages (BA), growth velocities (GV) and BMIs of patients during treatment were evaluated.
Results
On admission mean chronologic age (CA) and BA were 6.18 ± 2.1 years and 10.5 ± 2 years respectively. Five children have 46,XX karyotype, but one of them was reared as a male. Mean follow-up 4 ± 1.8 years. A significant difference was found between mean BA/CA on admission and at last visit ($P=0.002$; t: 4933), and between mean BA/CA the beginning of GnRHa-T and at last visit ($P=0.002$; t: 4453). Mean CA was significantly increased in girls than boys at the beginning of GnRHa-T (8.37 ± 0.9 vs 5.2 ± 1.5 years; $P=0.032$).
GV was 5.96 ± 2.2, 6.98 ± 2.9 and 4.77 ± 2.8 cm at the end of first (GV1), second (GV2) and third years of the therapy respectively. BMI was inversely correlated with GV1 (r: -0.818; $P=0.007$) and GV2 (r: -0.731; $P=0.039$). Mean CA at the beginning of GnRHa-T was negatively correlated with GV1 (r: -0.714; $P=0.047$) and GV2 (r: -0.927; $P=0.003$).

	On admission	At the beginning of GnRHa therapy	At last visit on therapy
Mean chronologic age (CA; years)	6.18 ± 2.1	6.78 ± 1.8	10.1 ± 2
Mean bone age (BA; years)	10.5 ± 2	11.2 ± 1.7	12.3 ± 2.1
Mean BA/CA	1.93 ± 0.6	1.7 ± 0.4	1.22 ± 0.2
Mean predicted height (cm)	157.6 ± 21		161.3 ± 11
Mean BMI–SDS	0.62 ± 0.67		0.77 ± 0.9

Conclusion
GnRHa-T should be considered for augmentation of linear growth in children with CAH complicated with CPP, particularly in children with not too advanced BA for CA.
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P803

Growth and pubertal development in adolescent male wrestlers
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Introduction
Adolescence is a crucial period for linear growth, and sports training during this time may have positive or negative effects on some physiological processes as growth. The purpose of this study was to evaluate the effect of intense training during somatic growth on the onset of puberty and growth development in adolescent wrestlers.
Description of methods/design
Fifty adolescent male wrestlers and 21 sedentary healthy male controls aged 13–15 years were selected. The wrestlers were active at a competitive level from five different wrestling schools. The maturity status of subjects and data about the anthropometric characteristics were evaluated. Serum levels of testosterone, DHEA-S, FSH, LH, prolactin, cortisol, IGF1, TSH and free thyroxine (fT_4) were determined.
Results
Anthropometric characteristics and puberty levels according to Tanner stage were similar in both groups. Sex hormones and cortisol, IGF1, prolactin levels did not differ statistically but TSH concentrations differed significantly between wrestlers and sedentary control groups ($P=0.015$).
Conclusion
The results suggest that training in adolescent male wrestlers did not significantly change resting sex hormones or alter the onset of puberty as determined by assessment of pubertal stages. The wrestlers had lower body fat and greater energy expenditure per week, there were no significant differences in height, weight, or BMI.
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P804

GH therapy and effect on ovarian function and morphology in short prepubertal SGA girls
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Background
GH receptors are present in ovaries and GH may have a physiological role for ovarian function and development.
Objective and hypothesis
The objective of this study was to examine pubertal development and ovarian growth and differentiation during GH therapy.
Methods
Clinical characteristics, reproductive hormones and ultrasonographic examination of the internal genitals were determined in 18 prepubertal girls during 3 years of GH therapy in a Danish sub-study of the North European SGA study (NESGAS), a multinational, randomised, longitudinal study of GH therapy in short prepubertal children born SGA.
Results
Median age at baseline was 4.91 years (4.51–7.22). Bone age advanced significantly during 3 years of treatment ($P=0.007$), but did not exceed chronological age. Uterine and ovarian volume increased significantly (1.05–1.72 ml, $P=0.033$ and 0.43–0.9 ml, $P=0.005$ respectively), but remained within the lower reference ranges. Ovarian follicles became visible in 69% compared to 27% before GH therapy ($P=0.025$). Precocious puberty was observed in one girl and another girl showed signs of a multicystic ovary.
AMH tended to cluster in the lower part of the reference range, but increased significantly during 3-year of treatment ($P=0.028$).
SHBG decreased during the first year of GH therapy ($P<0.001$) and remained low, while an increase in androstenedione and DHEAS was found during 3 years ($P=0.043$ and $P=0.005$ respectively). No cases of precocious pubarche were observed.
Inhibin B increased significantly during the first 3 years of treatment, but no significant changes in FSH, LH, estradiol or inhibin A were found.
Conclusions
GH treatment of short SGA girls can generally be considered safe, but as altered pubertal development and ovarian morphology was observed in 2 out of 18 girls, pubertal development and ovarian function should be monitored during GH therapy.

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P805

GH dynamics in oral glucose tolerance test in children and adolescents with tall stature

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Background
Oral glucose tolerance test (OGTT) is a step in the evaluation of children and adolescents with tall stature for documenting a possible autonomous GH secretion.
Aim
Assessment of GH dynamics in OGTT in children and adolescents with tall stature in various stages of pubertal development for documenting a possible autonomous GH secretion.
Method
Our study included 44 subjects, 18 girls and 26 boys, with age between 6.5 and 17.3 years diagnosed with constitutional tall stature. The inclusion criterion was height $\geq +2$ S.D. The exclusion criteria were: diabetes, thyroid dysfunction, Cushing's syndrome, hypothalamic–pituitary pathology, renal failure, medication that alters glucose/GH dynamics such as estrogen and thyroid hormones. The following parameters were assesed: historical and auxological data, clinical examination, laboratory evaluations: karyotype, thyroid function, androgens, IGF1, IGFBP3, prolactin, evaluation of other pituitary hormone deficiency, age bone, cardiac ultrasound, eye examination, CT/MRI of the hypothalamic–pituitary region. OGTT was performed with 1.75 g/kg of glucose po. Glucose and GH (CLIA method) were tested at 0, 30, 60, 90 and 120 min. Statistical analysis was performed using PASW Software version 18, 2010.
Results
GH suppression was different according to sex and pubertal stage. For boys in stage III and IV Tanner we observed GH values >1 ng/ml. For girls GH nadir was higher than for boys. The lowest value of GH both for girls and boys was at 90 min. Two patients with IGF1 $>+2$ S.D. for chronological age had normal levels of IGFBP3. In six patients with GH suppression >1 ng/ml, the levels of IGF1 and IGFBP3 where normal.
Conclusions
Because GH level is high at puberty compared to the level seen in adulthood data obtained in the oral glucose tolerance test in adulthood can not be extrapolated for children and adolescents. It is necessary to establish new cutoff for GH suppression in oral glucose tolerance test according to sex and pubertal stage both for normal height and tall stature.

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P806

Obesity and thyroid function in children: cross-sectional study

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Obesity and thyroid function in children – cross-sectional study.
Background
Obesity in children has been increasing dramatically, with a significant increase in cardiovascular and metabolic diseases risk. The role of thyroid dysfunction has been extensively analyzed in obese adults, but to a limited extent in children.
Aims
To estimate the prevalence of hyperthyrotropinemia in obese children and to analyze the influence of BMI–SDS and TSH in other metabolic variables.
Methods
Retrospective study with data from the first evaluation of obese children in our clinic. Demographic, anthropometric and metabolic variables were studied. Descriptive analysis consisted of frequencies distribution for qualitative variables and mean $\pm$ S.D. for continuous variables. For the association between BMI–SDS, thyroid function and other metabolic variables multiple linear regression models were used. A P value ≤ 0.05 was considered for statistical significance.
Results
We obtained data from 348 children with mean age of 11.7 ± 3.1 years and mean BMI–SDS of 2.9 ± 0.7. The prevalence of hyperthyrotropinemia was 8.9% and of homeostasis model assessment-insulin resistance (HOMA-IR) elevation was 69.3%. Children with hyperthyrotropinemia revealed fT_3 and HOMA-IR significantly higher than those with normal serum values. BMI–SDS was positively correlated with TSH and fT_4 but not with fT_3, when controlled for sex, age and pubertal stage. BMI–SDS and TSH were positively and independently correlated with HOMA-IR, but not with the lipids.
Conclusions
The prevalence of hyperthyrotropinemia was similar to that reported in other studies and appeared to be influenced by metabolic factors other than fT_3 or TRH. Children with hyperthyrotropinemia had significantly higher fT_3 and HOMA-IR. BMI–SDS was positively correlated with TSH, fT_4 and HOMA-IR. TSH and BMI–SDS were positively and independently correlated with HOMA-IR, which suggests that hyperthyrotropinemia might exacerbate insulin resistance in obese children.

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P807

Final height of a group of patients with congenital adrenal hyperplasia

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Introduction
The final height of children with congenital adrenal hyperplasia (CAH) seems to be compromised. That can happen because of the disease itself or because an excessive dose of corticoids used during treatment. This work evaluates the final stature SDS of a group of patients with CAH and correlates it with 17-hydroxyprogesterone levels (17OHP) and corticoid dose at 10 years of age and at puberty onset.
Methods
Selection of children with CAH diagnosed from 1983/08/01 to 1995/08/01, followed in an outpatient clinic of paediatric endocrinology, with all necessary data in their clinical files: height, weight, 17OHP and corticoid dose at 10 years of

age, puberty onset and at the last medical evaluation. Data was analysed in SPSS 20.0, with descriptive statistics, Wilcoxon test, Spearman's correlation coefficient and Mann-Witney *U* test (significance level: 0.05).
Results
There were 19 children with CAH: 8 boys; 11 girls (13 were diagnosed in the first year of life). The final height SDS was −2.07 (−4.01 to −0.55). It was different from the familiar height SDS: −1.33 (−2.34 to −0.22; $P=0.003$). At puberty onset, their height SDS was 0.33 (−2.6 to 5.14); at 10 years of age, their height SDS was 0.29 (−1.69 to 3.09). The final height SDS had no statistically significant correlation with 17OHP and corticoid dose used at puberty onset, at 10 years of age or at the last clinical evaluation. The difference between the final height SDS and the pubertal height SDS was positively correlated with corticoid dose used at puberty onset ($r=0.466$; $P=0.044$); that difference was negatively correlated with 17OHP at puberty onset ($r=-0.643$; $P=0.004$).
Conclusion
In this group of children the final height SDS was inferior to the familiar, because of the poor growth after puberty onset. There wasn't any correlation with 17OHP and corticoid dose used during treatment.

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P808

Sex steroid priming in differential diagnosis between idiopathic GH deficiency and constitutional delay of growth and puberty

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Priming with sex steroids prior to stimulation tests for the diagnosis of GH deficiency (GHD) in peripubertal years remains controversial, though some evidence suggests its utility in improving the specificity of GH testing for the distinction between idiopathic GHD (IGHD) and constitutional delay of growth and puberty (CDGP). However, few data are available on the final height (FH) of untreated CDGP patients. In order to better understand the usefulness of priming, we analyzed the FH reached by two groups of consecutive IGHD or CDGP patients.
Study
Forty-five short children (30 males and 15 females; age 8, 9–16 years) underwent sex steroid priming (i.m. testosterone 100 mg; 1–2 mg oral estradiol) before GH stimulation test and were diagnosed with IGHD (25 patients) or CDGP (20 patients) depending on the results of testing. Only IGHD patients were treated with rhGH (0.025–0.035 mg/kg daily). All patients were followed-up until FH.
Results
Mean GH peak following testing was significantly lower in IGHD than in CDGP ($P<0.0001$). Mean IGF1 SDS was also significantly lower in IGHD ($P<0.01$): in particular, all children with IGF1 SDS <-2.4 (24% of total) were diagnosed with IGHD. Mean initial height SDS (IHSDS) was similar between the two groups, while target height SDS (THSDS) was significantly lower in IGHD ($P<0.05$). Both groups of patients reached a FH not statistically different from their TH.
Conclusions
i) Frankly low levels of IGF1 are highly suggestive of GHD, making the use of sex steroid priming unnecessary. ii) The significantly lower THSDS of IGHD patients suggests the contribution of inheritable factors in this category of children. iii) Our data support the usefulness of sex steroid priming in improving the ability of GH stimulation testing to differentiate IGHD from CDGP, thus avoiding an unnecessary expensive treatment.

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P809

Importance of gastroenterologist in successful recovery of anorexia nervosa patients

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Presumption, nausea, bowel distension, abdominal pain and early satiety are very common in patients with Anorexia nervosa (AN). These problems may give rise to significant medical complications and may contribute to increased difficulties with refeeding and weight restoration. The aim of our study was to evaluate the influence of gatrointestinal treatment on weight gain in AN patients. Esophagogastroduodenoscopy with gastric mucose biopsy were performed in 19 AN patients (DSM-IV criteria) with digestive problems (mean age 22.4±0.7 years, BMI 15.9±2.3 kg/m^2) Patohistological finding confirmed chronic gastritis in all AN patients and in ten patients *Helicobacter pylori* (HP) infection was found**.** The same examination was pereformed in 19 aged matched controls (25.2±0.7 years, BMI 19.8±0.4 kg/m^2). No significant difference was found between HP positive and negative AN patients at the beginning of gastrointestinal treatment in BMI (15.86±1.4 vs 16.1±1.17 kg/m^2, $P>0.05$), as well as in serum leptin levels (1.69±0.56 vs 2.38±2.1 ng/ml, $P>0.05$). However, significant differences in BMI and serum leptin levels in AN vs controls(BMI 15.98±1.3 vs 19.8±0.4 kg/m^2, $P<0.01$; leptin 2.48±0.5 vs 9.1±1.6 ng/ml, $P<0.01$) were found. All AN patients were on hypernutrition and gastrointestinal therapy. After 6 months, in AN patients, significant difference in BMI and serum leptin concentrations (BMI 15.98±1.3 vs 18.0±1.3 kg/m^2, $P<0.001$, leptin 2.48±0.5 vs 4.9±0.7 ng/ml, $P<0.001$) were found.
Conclusion
Knowledge and treatment of gastrointestinal complications may be of critical importance in successful nutritive recovery in AN patients.

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P810

Primary amenorrhea aetiologies: results from a monocenter study

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Introduction
Compared to secondary amenorrhea, primary amenorrhea is deemed to be a rare condition. Our aim is to study its annual frequency during a long period of time, and to analyze its different aetiologies in an Endocrine Department.
Methods
All patients referred for primary amenorrhea between 1980 and 2012 were studied. We took in account personal and family history, clinical examination, hormonal, cytogenetic and immunological assessments, and radiological explorations.
Results
155 cases were collected in 32 years = 4.8 case/year. Their mean age at diagnosis was 20.4 years. For different aetiologies, we found endocrine origin in 151cases = 97.5% and gynaecological causes in four patients (2.5%). The last ones were related to Rokitanski Kuster syndrome.
Among endocrine aetiologies, hypothalamic and pituitary causes were observed in 84 = 55.7%, ovarian causes in 54 = 35.7%, and male pseudo hermaphrodisms in 13 = 8.6%.
Conclusion
In our study it appears that primary amenorrhea is a relatively rare consultation motive. The consultation was late in all cases. Gynaecological causes are rare. Among endocrine causes, hypothalamic and pituitary causes are prevailing. For ovarian causes, chromosomal causes are the most frequent. Male pseudo hermaphrodisms account for 8.6%.

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P811

Serum levels of 25(OH)-vitamin D and adipokine's profile in obese children and adolescents

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Background
Low-serum concentrations of 25(OH)-vitamin D are associated with insulin resistance in adults. Recent *in vitro* studies have suggested that vitamin D may play a role in the regulation of adiponectin, leptin and resistin; since all these adipokines are related to insulin sensitivity modulation, they might represent a link between vitamin D status and insulin resistance.

Aim
To identify possible correlations between 25(OH)-vitamin D serum levels and adipokine's profile in obese children and adolescents.
Material and method
Serum 25(OH)-vitamin D levels were assessed in 46 obese children and adolescents (age 14.3±2.2 years) compared to a control group of 30 age-matched healthy non-obese children (immunochemiluminiscence). We measured in both groups fasting insulinemia, HOMA-index, plasmatic levels of adiponectin, leptin and resistin.
Results
85.5% of subjects were vitamin D deficient (serum vitamin D <30 ng/ml; 87% in the obese group, 83.3% in the control group). The plasmatic levels of 25(OH)-vitamin D were not different between groups. The adiponectin levels were significantly lower and the leptin and resistin levels were significantly higher in the obese group compared to the control group.
25(OH)-vitamin D was negatively correlated to fasting insulinemia ($r=-0.324$, $P=0.036$) and to plasmatic levels of leptin ($r=-0.363$, $P=0.013$); in multivariate regression analysis the only parameter that influenced vitamin D status in obese children was leptin.
We found no correlation between plasmatic levels of 25(OH)-vitamin D and the plasmatic levels of adiponectin or resistin.
Conclusion
We found a deficit of vitamin D in a large majority of selected Romanian children. Vitamin D deficit in obese children is related to hyperinsulinemia, their association being explained by a parallel variation with plasmatic levels of leptin.

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P812

Hepatic function in Berardinelli–Seip patients

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Metabolic liver dysfunction can be a causative factor for morbity and mortality in Berardinelli–Seip syndrome patients. We evaluated hepatic function in 29 Berardinelli–Seip patients. Diabetes mellitus (DM) was present in 23 of them. We analyzed biochemical parameters including AST, ALT, GGT, ALP levels and liver non invasive imaging aspects. The liver was graded as normal, mild, moderate, or severe hepatic steatosis.
Results
We could not find significant correlation of DM diagnosis and dysfunction hepatic and steatosis. However we observed that time of diagnosis in these Berardinelli–Seip individuals showed relation to degrees of steatosis ($P=0.004$). It seems that the duration of clinical disease is more important than DM diagnosis regarding steatosis severity in these individuals.

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P813

An adolescent girl with hypothyroid coma due to autoimmune thyroiditis

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Profound hypothyroidism leading to coma has not been reported in adolescents.
Case presentation
A 13-year-old adolescent girl presented with coma. Mother reported fatigue, increased sleepiness, deterioration of school performance, apathy, secondary amenorrhea, change in voice, and weight gain for 5 months. No history of dyspnea, palpitations or chest pain, drug intake, trauma, or any systemic illness. No family history of endocrine disorders was reported. Mother reported that the girl lost consciousness after 30 min of feeling dizzy. She had hypothermia (36 C), hypotension (BP=90/55 mmHg), and bradycardia (50/min). She was comatosed (GCS=8/15) with periorbital edema, loss of the lateral eyebrows, dry skin, and large smooth symmetrical firm (40 g) goiter. Her TSH=417 mU/l and FT_4 of 1.7 pmol/l (normal 11–19 pmol/l) and antimicosomal antibody (AMA) titer of 1:1800 confirmed the presence of severe hypothyroidism due to autoimmune thyroiditis. Thyroid ultrasonography revealed bilaterally enlarged thyroid lobes with heterogenous echopattern and multiple nodules. MRI of the sella turcica revealed global diffuse enlargement of the pituitary. She received intravenous T_3 therapy that regained her consciousness in 10 h, followed by intake of L-thyroxine 100 μg daily. Vigor returned and voice improved within 2 weeks. FT_4 and TSH were normalized in 4 weeks. Pituitary size was normalized in the follow up MRI after 6 months.
Discussion
In this case presentation of hypothyroid coma is preceded by lethargy, depression, weakness, forgetfulness, cold intolerance and menstrual disturbance of insidious onset that was overlooked for 5 months. The presence of goiter, periorbital and facial edema and absence of lateral eyebrows were prominent. Aggressive therapy with intravenous thyroxine and external warming lead to excellent prognosis.
Conclusion
This case raises the awareness of physicians to include hypothyroid in the differential diagnosis of coma in this age group.

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P814

Features of hyperprolactinemia syndrome in children

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Aim
Evaluate the data of anamnesis, clinical-laboratory parameters, magnetic resonance imaging (MRI) findings, response to treatment in children with hyperprolactinemia (HProlact).
Methods
We analyzed retrospectively 16 patients in the endocrinological department of University hospital (Minsk) with HProlact over 2004–2012 years. Boys (B) 6 (37.5%) (stage on Tanner 1 – 1 (17%); stage 2–3 – 3 (50%), stage 4 – 2 (33%), age at diagnosis 11.4–15.9 years; girls (G) 10 (62.5%) (stage on Tanner 1–3 (30%), stage 2–3 – 2 (20%), stage 4 – 5 (50%), age at diagnosis 0.8–16.4 years. We examined BMI; the levels of prolactin, thyroid-stimulating hormone (TSH), free thyroxine; MRI. The results were processed using the Statistica 6.1.
Results
In anamnesis B: breast increasing 4 (66%), delayed puberty 1 (17%); G: precocious thelarche 3 (30%), dismenorrhoea 2 (20%), galactorrhoea 1 (10%), delayed puberty 1 (10%), headache 1 (10%).
Prolactin B 49.8±3.9, G 48.9±4.2 (3–27.7 ng/ml) ($P=0.6$). Subclinical hypothyroidism was in 2 (33%) B and 4 (40%) G with TSH levels 6.4±1.8 and 5.7±1.1 (0.23–3.4 μIU/l) ($P=0.2$). The remaining B and G were euthyroid. MRI confirmed the presence of microadenoma in 4 (66%) B and 5 (50%) G.
4 (66%) B and 4 (57.2%) G received treatment with bromocriptine (1.25–2.5 mg/day). 3 (42.8%) G were treated with cabergoline (500 μg/week). Normal prolactin levels were noted in all B after 8.7±3 month of treatment. G which received bromocriptine normal prolactin were after 3±1.7 month, cabergoline – 4±2 ($P=0.1$). Tumor decreasing from 5 till 3 mm by MRI were in all B and G after 9.6±2.5 months and 4.7±2.5 respectively.
Conclusions
HProlact syndrome is rare in children. All cured patients with HProlact demonstrated a good response to medical treatment by normalization of prolactin values and decreasing of tumor.

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P815

Growth disorders in Greece: baseline data from a multicentre observational study (GENESIS)

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Aim
The Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS) is an open-label multinational observational study which collects information on management, clinical outcomes and treatment safety of children with growth disorders. Here we present descriptive data from the Greek cohort.
Methods and results
In Greece, 211 children (44.5% females, 136 naive to GH treatment at study entry and 18 not GH-treated) have been enrolled, after providing informed consent, in eight investigational sites, over 6 years (2005–2011). GH deficiency (GHD, *N* 177) and Turner syndrome (TS) (*N* 20) were the main diagnoses upon enrollment. GHD was diagnosed with higher frequency in males than females (63.5 vs 36.5%). In patients where pubertal stage had been recorded, 70.4% of females (GHD 67.9%, TS 76.5%) and 78.3% of males were pre-pubertal (Tanner B1 and G1 respectively). The most frequently performed tests to confirm the need for GH administration were glucagon (40.0%) and clonidine (32.0%). When a combination test was performed glucagon and clonidine were paired most frequently (50%). The mean max GH peak was 6.1 ± 3.1 and 12.4 ± 8.2 μg/l for patients with GHD and TS respectively. Baseline characteristics to be depicted in table.
Conclusions
In the Greek cohort of GeNeSIS, GHD is the most frequent cause for GH treatment, followed by TS. While the latter is diagnosed earlier, bone age to chronological age gap is numerically smaller and a higher GH initiation dose is administered. The results should be interpreted in the context of an observational, ongoing study.

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P816

Predicting growth response among Egyptian idiopathic isolated GH deficient children

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Objective
To determine the Predictors of growth response to GH treatment in a group of isolated idiopathic GH (GH).
Patients and methods
477 GH deficient (GHD) children with GH therapy were included in the study. Patients were followed up for a minimum of 1 year up to 6 years. Multiple linear regressions were done to identify predictors of growth response to rhGH in the first 4 years of treatment.
Results
In the first year, three significant predictors of growth were identified: GH peak (ln (μg/l)), age of onset of therapy and target height-height SDS. In the 2nd and 3rd years of therapy, GV was both significantly and positively correlated to the GV (cm/year) of the previous year.
Conclusion
We conclude that, prediction models offer a valuable tool for individualization and assuring adherence to rhGH and thus a cost effective treatment, which is the ultimate goal of GH therapy.

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P817

Williams syndrome: report of a case

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Introduction
Williams–Beuren syndrome is a rare genetic condition with clinical manifestations that include a distinct facial appearance, cardiovascular anomalies that may be present at birth or may develop later in life, idiopathic hypercalcemia, and a characteristic neurodevelopmental and behavioral profile.
Case report
We present a particular case of a 3.5 years old boy, born SGA at 39 weeks of gestation with neonatal hypoxia, diagnosed at 1.6 years old with Williams syndrome (FISH analysis: microdeletion 7q11.23 (ELN/D7S613-), after multiple neuro-psychiatric evaluations for developmental delay and cognitive deficits.
Clinical exam
Normal stature, ponderal hypotrofia, facial dysmorphism: bilateral epicanthal folds, hypertelorism, microcephaly, short upturned nose, long philtrum, wide mouth, full lips, dental malocclusion and widely spaced teeth, micrognathia, blue–green eyes, stellate irises, lacrimal ducts imperforation; sunken chest, normotensive, systolic murmur, retractile testes, congenital fimosis, G1P1 Tanner stage; mild-moderate psychomotor delay, lower limb spastic hypertonia, hyperreflexia. Psychological examination: hyperkinetic, with mild heteroaggressive elements, attention deficit disorder, easily distracted; borderline intelligence. Echocardiography: mild supravalvular aortic stenosis, isolated septal hypetrophy. Normal renal function and ultrasound. Normal thyroid function, upper normal limit serum calcium (10.1 mg/dl, NV = 8.6–10.4 mg/dl), normal glycaemia.
Conclusion
Although the patient did not develope any endocrine feature by the the time of presentation we report this case to illustrate a complex multisystem medical condition that requires a multidisciplinary team depending on the specific phenotype manifestations. Rehabilitation programs and educational interventions improve the prognosis and the social adjustment of the patient. This case needs family support and periodicallly referrals to pediatric neurologist and psychiatrist, cardiologist and/or cardiac surgeon, endocrinologist (for calcium and vitamin D levels, thyroid function, glucose tolerance testing, gonadal function), genitourinary tract ultrasound, assessments for visual problems or hearing loss.

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P818

Case report: two patients with Di George syndrome with different diagnostic peculiarities

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Introduction
Di George syndrome is a genetic disorder caused by deletion of chromosome 22. The main features are congenital heart defects, absence or hypoplasia of thymus (with consecutive immunodeficiency and infections), hypoparathyroidism with hypocalcaemia, gastrointestinal problems, delayed psychomotor development, craniofacial abnormalities, tendency to develop seizures and psychiatric disorders.
Case report
We present the case of two patients with different peculiarities regarding age at diagnosis, clinical features that arose suspicion and lead to genetic testing. First case a 13.6 years old boy is diagnosed with Di George syndrome in FISH analysis after presenting for over 1 year muscle cramps in lower limbs and constipation due to hypocalcemia, multiple respiratory tract infections, mild facial dysmorphism (considered familial and did not raise any suspicion until now), scoliosis, seizures, attention deficit hyperactivity disorder. Laboratory tests: hypoparathyroidism – hypocalcemia, euthyroidism, negative for autoimmune thyroid disorder, normal serum cortisol and ACTH; normal CBC. No thymus evaluation or immunologic tests yet available. Normal renal and cardiac ultrasound.

Second case an 6-year-old girl with genetic confirmation of 22q11.2 microdeletion at 4 years old. Clinical presentation: normal stature, overweight (+3.34 S.D.), mild craniofacial dysmorphism: long face, tubular nose, mild hypoplasia of nasal wings, low-set ears posterior rotation, hypernasal voice. She was diagnosed with epilepsy at 11 months. Psychological evaluation: delay in expressive language and psychosomatic development, polymorphic dislalia. Laboratory tests: normal calcemia, euthyroidism. Normal renal ultrasound.
Conclusion
Common aspects for both patients are euthyroidism, developmental delay and learning difficulties, low performance intelligence quotient, absence of thymus disorders but recurrent infections in both patients' medical history require further immunology tests to be assessed. Both are children with special needs requiring continual care and supervision (because of metal retardation, seizures, neurological and psychiatric disorders).

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P819

Egyptian GH deficient patients: demographic, auxologixcal characterization and response to GH therapy

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Objective
To study the growth response to GH treatment in GH deficient patients. Also to report the possible side effects emerging during treatment with GH.
Patients and methods
477 GH deficient (GHD) children were included in the study. All patients received biosynthetic GH therapy at a dose of 20 IU/m^2 per week. Patients were followed up for a minimum of 1 year and up to 6 years as anthropometric assessment was performed every 3 months, while thyroid profile was followed every 6 months. Student's *t*-test was used for analysis of two quantitative variables**.**
Results
Patients with complete GHD and multiple pituitary hormone deficiency (MPHD) were significantly shorter as expressed in their height SDS, target height–height (SDS) and a more bone age delay ($P<0.05$). Patients with complete GHD showed better growth response compared to those with partial GHD in the first 2 years of therapy.
Conclusion
We conclude that anthropometric assessment is the corner stone in GHD diagnosis and follow up, where catch up growth occurs in the first 2 years of therapy followed by a plateau. GH therapy is generally safe.

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P820

Male pseudo hermaphrodism due to the association of two very rare conditions: a deficit in 17β-hydroxysteroid dehydrogenase type 3, and a chimerism

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Introduction
In medical practice ambiguous genitalia is a relatively are condition. The combination of two causes in genital malformation is exceptional. Our aim is to describe a person having an abnormal karyotype with a chimerism (46,XY/46,XX) and a deficit in 17β-hydroxysteroid dehydrogenase 3 (17β-HSD): an enzyme in the testes that transforms D4 Androstenedione to testosterone and androstenedione to dihydro testosterone or DHT.
Case report
X aged 21, whose parents are cousins, educated as a girl, came for primary amenorrhea. Clinical examination showed deep voice, mild ambiguous genitalia, clitoro-megaly and two testes (one in the scrotum and the second highly situated). The breast was Tanner's stage 1, body hair repartition looks like a woman's. Hormonal assessment showed high testosterone for a woman and low for a man (9.25 nmol/l) and very high D4A (13 ng/ml). On ultrasound female structures were absent. Pelvis MRI confirmed both testes and seminal vesicules. The karyotype showed a mosaic: 46,XY (96%) and 46,XX (5%) or chimerism.
Conclusion
In our case, the male pseudo hermaphrodism is apparently due to the combination of two exceptional abnormalities. The first one is a chimerism in which the person has the male chromosomal complement (XY), but some cells have the female chromosomal complement (XX). The second is also a very rare as partial deficit in 17β-HSD3, i.e. enzyme that transforms D4A to testosterone and androsterone to DHT. Both exceptional situations can lead to ambiguous genitalia.

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P821

Results of investigation of children and adolescents (boys) with different disorders of puberty development in Bukhara city and four districts (Republic of Uzbekistan)

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Aim
To find and study character of clinical disorders of children and adolescents (boys) with different disorders of puberty development.
Materials and methods
During April–May 2012 we examined 521 children and adolescents (boys) in Bukhara city and four Bukhara districts schools. 143 of them were underwent general clinical examination as well as biochemical and hormonal investigations (levels of LH, FSH, GH, testosterone, sex-steroid ass. globulin, prolactin, cortisol, TSH, T_4, etc.), roentgenologic examination (Turkish saddle CT and MRI and hand roentgenogram), clinical ultrasound of the thyroid and sex organs, anthropometric examination, having their sexual development stage by Tanner assessed. Mean age of boys was from 11.0 to 16 years.
Results
Examination of 521 boys allowed diagnosing a number of different disorders of puberty development in 143 boys (29.4%). By character of pathology the examinees were divided into three groups. The 1st group included 106 patients from 143 (74.1%) with delay of puberty, the 2nd group including 13 patients (9.09%) with retardation of puberty and growth, the 3rd group including 11 patients (7.7%) with gynecomastia (one of boys having obesity), the 4th group – five patients (3.5%) having micropenis + obesity, the 5th group – two patients (1.4%) having varicocele, the 6th group – 2 patients (1.4%) with anorchia, the 7th group – two patients (1.4%) having cryptorchidism, the 8th group – and one patient (0.6%) having precocious puberty.
Conclusions
i) Among disorders of puberty development in teenagers more frequent found delay of puberty (74.1%). ii) There are different disorders of puberty development was established: retardation of puberty and growth (9.0%), gynecomastia (7.7%), micropenis + obesity (3%), varicocele (1.4%), cryptorchidism (1.4), and precocious puberty (0.6%).

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P822

Hormonal results of investigation of adolescents (boys) with delay of puberty

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Aim
To study hormonal results of investigation of adolescents (boys) with delay of puberty.
Materials and methods
We choice 32 teenagers from 106 boys with symptoms of delay of puberty development in the age of 15 years old. They were underwent general clinical examination as well as biochemical and hormonal investigations (levels of LH, FSH, GH, testosterone, prolactin, cortisol, TSH, T_4, radio-immunological lab of The Center of Endocrinology, PhD, Abdurakhmanova A.M.).
Results
The hormonal investigation of 32 boys allowed diagnosing a number of different disorders. The mean level of LH was 2.8 mME/l (5.1 ± 0.6), FSH – 3.4 mME/l (6.1 ± 4.9), TSH – 1.82 mME/l, total testosterone – 8.9 ng/ml (mean 6.24 ng/ml), prolactin – 7.4 nmol/l (5.8), cortisol – 289.25 nmol/l (250–720), and T_4 – 17.4 ng/ml.

Conclusions
i) The hormonal investigation of teenagers with mean age 15 years old submitted, that most of our patients (29 from 32) had hypogonadotropic hypogonadism (90.6%) and ii) only one patient had hypergonadotropic hypogonadism (3.1%).

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Pituitary–Basic (*Generously supported by IPSEN*)

P823

Gene expression profiling of familial and sporadic pituitary adenomas

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Background
Familial isolated pituitary adenoma (FIPA) is recently identified autosomal dominant condition with incomplete penetrance. Heterozygote mutations have been identified in the aryl-hydrocarbon receptor interacting protein (AIP) gene in 20–30% of FIPA families. AIP mutation positive patients have distinct phenotype: the disease is occurring at a younger age and have more aggressive tumours.
Aims
The aim of this study was to perform comparative gene expression analysis of AIP positive, AIP negative and sporadic tumours to discover the genes/pathways responsible for the AIP positive phenotype and to understand the underlying molecular mechanisms involved in the pituitary tumorigenesis.
Methods
We have performed gene expression analysis on normal pituitary, sporadic GH-secreting adenomas, AIP positive and AIP negative familial somatotroph adenomas (five samples of each category) using the Affymetrix human Gene Chip HG-U133 Plus 2.0 array. Data analysis was carried out in the statistical 'R' environment. Ingenuity pathway analysis (IPA) tool was used for pathway analysis. Expression of the five selected genes from microarray analysis was validated by quantitative reverse transcriptase PCR.
Results
We have identified a large number of differentially expressed genes in pituitary adenomas compared to normal pituitary. In addition, a small number of genes differ in their expression levels between familial AIP positive and sporadic adenomas. These genes are involved in epithelial-to-mesenchymal transition (EMT), extra-cellular matrix (ECM) remodelling and cellular invasion. QRT-PCR data of the increased expression of mesenchymal marker, invasive markers and the decreased expression of epithelial markers were consistent with the microarray data.
Conclusion
These results indicate that these transcriptional changes likely reflect the clinically seen more aggressive phenotype in AIP positive patients. In pituitary tumorigenesis EMT likely occurs within a specific genetic context and may be related to their increased local invasion and more aggressive behaviour. Therefore, different pathways in pituitary adenoma progression exist.

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P824

Role of filamin-A in the regulation of SST2 receptor localization and signalling in tumoral GH-secreting cells

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Somatostatin (SS) binds to different SS receptors (SSTR1–5) and SS analogues are the first choice medical treatment of GH-secreting pituitary adenomas (GH-omas). A subset of patients is resistant to SS, although the mechanisms involved in SS resistance are not fully understood. Recent studies identified specific protein–protein interactions as determinant in the regulation of receptor anchoring and signalling. Filamin A (FLNA) is a widely expressed cytoskeleton protein that, through its scaffolding properties, affects the intracellular signalling and trafficking of a number of receptors. Based on our recently published data, FLNA is crucial for D2 receptor expression and signalling in lactotrophs cells. Since SSTR2 was recently found to associate with FLNA, the aim of this study was to investigate the role of FLNA in SSTR2 signalling and targeting in human GH-omas and GH3, a rat pituitary GH-secreting cell line.
We studied FLNA expression in GH-omas ($n = 10$) by western blotting (WB) and its role in GH3 by gene silencing technique. Confocal microscopy was used to evaluate receptor SSTR2 localization and WB to evaluated cyclin CD1 and SST2R expression.
In all GH-omas FLNA was expressed at variable levels, without any significant correlation with the clinical phenotype. In GH3 cells FLNA gene silencing did not induce changes in SSTR2 total levels. Similarly, this manipulation did not affect receptor localization at the plasma membrane. On the contrary, the reduction in cyclin D1 levels induced by the selective SSTR2 agonist (Bim23120) in GH3 was abolished in FLNA silenced cells.
These data suggest that FLNA might be implicated in intracellular signalling of SSTR2 by mediating at least some of its antiproliferative effects. In contrast, FLNA does not appear to be necessary for receptor expression and localization at the plasma membrane.

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P825

***Drosophila melanogaster* as a model organism to study aryl hydrocarbon receptor interacting protein gene function**

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Introduction
Loss-of-function mutations in *AIP* are associated with familial isolated pituitary adenoma, often leading to gigantism due to invasive GH-secreting pituitary adenomas. One challenging problem in the management of patients carrying a missense *AIP* variant is to determine whether the missense variant is a disease-causing mutation or not. As most of the molecular mechanisms involved in the control of growth and the cell cycle are well-conserved, we propose to utilise the fruitfly to determine putative disease-causing human variants. The *Drosophila melanogaster AIP* orthologue gene CG1847 codes for a protein with a similar size and structure.
Methods
We created CG1847 deficiency either via RNAi knockdown (using two different RNAi-lines) or via knocking out with the help of imprecise excision of a P-element located inside the 5′-UTR of *CG1847* gene.
Results
When a universal driver (actin) was used to express the *CG1847* RNAi, no viable adult offspring were observed, indicating that complete AIP-knockdown is lethal. To confirm these results with a different approach, we generated imprecise excisions of a P-element and deletion of a 1497 bp fragment, creating a loss-of-function mutation of *Drosophila AIP* ($CG1847^{exon1,2}$). This mutant is lethal in males (females are viable being heterozygotes for this $CG1847^{exon1,2}$ mutation).
Conclusions
We have demonstrated that total deficiency of CG1847 leads to lethality in fruitfly similar to results in AIP-KO mice, confirming that CG1847 is an essential gene for fly development. In our future experiments we will attempt to rescue the fruitfly $CG1847^{exon1,2}$ mutant with human AIP, and then we will make mutants containing different human AIP missense variants. These experiments should ultimately test the degree of functional conservation between fly and human, and help to determine whether the missense variant is likely to be a disease-causing mutation or not.

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P826

The role of GLI1 in pituitary tumor formation and pituitary cell survival

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The transcription factor and proto-oncogene GLI1 is the terminal mediator of the Hedgehog signalling pathway which is involved in developmental processes, stem cell maintenance and cell proliferation. Although the pathway is mainly active during embryogenesis and tissue repair, it is frequently reactivated in several cancer types.
Based on these findings we investigated the potential role of GLI1 in the pathogenesis of pituitary adenomas. GLI1 expression was studied in 30 human pituitary adenomas by qRT-PCR. Additionally, mRNA expression levels of stem cell marker SOX2, cell cycle regulator TP53, proliferation marker Ki67 and superoxide dismutase (SOD) 1 were determined. The threshold of GLI1 expression was set to be 1% of the GAPDH copy number level. Furthermore, the murine pituitary adenoma cell line AtT-20 was treated with the GLI1 antagonist GANT61 and cell viability was evaluated.
19 out of 30 human pituitary adenomas (63%) showed a GLI1 overexpression of various extent. GLI1 expression correlated with the expression of SOX2 ($P<0.001$, $r=0.5813$), TP53 ($P<0.001$, $r=0.6111$), Ki67 ($P=0.0385$, $r=0.3798$) and SOD1 ($P<0.001$, $r=0.5889$). Expression levels of all the above mentioned genes exhibited also a significant correlation among each other creating a network of cell cycle regulators and stem cell factors potentially involved in the pathogenesis of pituitary adenomas. Of note, survival rates of AtT-20 cells were highly decreased when treated with GLI1 antagonist GANT61. Cell survival was reduced by 50% (24 h) and 75% (48 h), respectively, upon incubation of cells with low concentrations (5 μM) of GANT61 and could be further decreased at a concentration of 20 μM resulting in complete cell death after 48 h.
In conclusion, our results suggest that GLI1 is potentially involved in the pathogenesis of pituitary adenomas by modulating adult stem cell fate or tumor-initiating stem cell function in the adult pituitary gland and its neoplasms.

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P827

cAMP exerts proliferative and anti-proliferative effects in pituitary cells of different types by activating both cAMP-dependent protein kinase A and exchange proteins directly activated by cAMP

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cAMP is implicated in the inhibition or stimulation of proliferation depending on cell type. The activation of cAMP–PKA pathway generates proliferative signals in GH-secreting adenomas whereas this effect is not present, or even opposite, in non-functioning pituitary cells (NFPA). Although cAMP effects were initially attributed to PKA activation, recently the discovery of two cAMP-activated guanine nucleotide exchange factors (Epac1,2) was proposed as a novel mechanism for governing signalling specificity within the cAMP cascade. The aim of the present study was to investigate the effects of cAMP in different pituitary cell types on cell proliferation and to determine the specific role of PKA and Epac in mediating these effects.
We tested the effects of different cAMP analogs (PKA-selective, Epac-selective or non selective) on cell proliferation, evaluating also the expression of cyclin D1/D3 and the cyclin dependent kinase inhibitor p27, in different pituitary adenomas (GH-, PRL-secreting or non secreting adenomas) and in appropriate cell lines (GH3, MMQ and HP75).
We found that non selective cAMP analog caused a 50% stimulation of somatotroph cells proliferation, whereas they exerted an opposite inhibitory effect on lactotrophs (−55%) and non-functioning (−58%) pituitary cells, these data being obtained also in the corresponding cell lines and confirmed by the expression of CD1, CD3, p27 proteins. Moreover, stimulatory and inhibitory effects induced by cAMP analog were mimicked by the PKA- and Epac-selective cAMP analogs.
In conclusion, we demonstrated that cAMP exerted opposite effects on proliferation in different pituitary cell types and that these effects are mediated by both PKA and Epac through the activation of different pathways, i.e. CREB and Rap1 respectively.

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P828

Expression of somatostatin receptors, $SSTR_{2A}$ and $SSTR_5$, in 108 pituitary adenomas, using immunohistochemical detection with specific MABs

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Background
Medical treatment of pituitary adenomas with somatostatin analogs depends on tumor type and somatostatin receptor expression. Using polyclonal antibodies, their immunohistochemical (IHC) detection gave conflicting results. Therefore, we studied the IHC expression of $SSTR_{2A}$ and $SSTR_5$ using two specific MABs in five types of pituitary adenomas.
Methods
$SSTR_{2A}$ and $SSTR_5$ expression was studied using two MABs (clone UMB1 and UMB4 for type 2A and 5 respectively) in 108 pituitary adenomas classified into five types: GH ($n=60$), ACTH ($n=15$), FSH/LH ($n=23$), PRL ($n=7$) and TSH ($n=3$). A comparative study was performed using two fixatives (Bouin-Hollande and Formol-Zinc) and two technical procedures (manual and automated IHC). GH adenomas were classified as pure or mixte GH/PRL, densely (DG) or sparsely granulated (SG) or according to the percentage of SSTR immunoreactive cells (<25%; 25–75%; >75%). In the other types of adenomas, the SSTR expression was considered as positive when more than 5% of the cells were immunoreactive.
Results
Whatever the fixatives, the techniques or the antibodies used, only specific membrane staining, without cytoplasmic reaction, was observed. Almost all GH adenomas expressed $SSTR_{2A}$ (93.3%) and $SSTR_5$ (81.6%) with high expression (>75%) in 53.6 and 36.5% respectively. No significant difference of $SSTR_{2A}$ and $SSTR_5$ expression was observed between DG and SG, or between pure and mixte GH/PRL adenomas. Moreover, $SSTR_{2A}$ was also expressed in 100% of TSH adenomas, and weakly expressed in 26% of FSH/LH adenomas, ACTH or PRL adenomas being negative. $SSTR_5$ expression was noted in 66% of TSH adenoma but only in 20% of ACTH adenomas, LH/FSH and PRL adenomas being negative.
Conclusion
$SSTR_{2A}$ and $SSTR_5$ were only highly expressed in GH and TSH adenomas. The IHC detection of SSTR with MABs is a reproducible and specific method which can be performed in everyday practice and could be helpful to predict post-operative treatment with somatostatin analogs, in particular in ACTH adenomas.

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P829

mTOR mediated IGF1 proliferative effects on a rat pituitary GH/PRL secreting pituitary adenoma cell line

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IGF1 represents an important growth factor in pituitary physiology and pathology, also mediating the negative feed-back mechanisms on somatotroph axis. It has been previously demonstrated that IGF1 promotes cell viability in primary cultures of human non functioning pituitary adenomas, by a mechanisms involving, at least in part, mTOR signaling, which is involved in many pathways controlling proliferation. We aimed at investigating whether mTOR may mediate IGF1 effects also in an *in vitro* model of GH/PRL secreting pituitary adenoma, the rat GH3 cells. Therefore, GH3 cells were incubated in the presence or in the

absence of 100 nM IGF1 with either Everolimus, an mTOR inhibitor, or NVP-BEZ235, a PI3K/mTOR inhibitor at concentrations ranging from 10 to 500 nM for 72 h. We found that the lowest Everolimus concentration that significantly inhibits cell viability is 25 nM, with a cell viability reduction of 50% ($P<0.01$). The lowest NVP-BEZ235 concentration that significantly inhibits cell viability is 50 nM, with a cell viability reduction of 40% ($P<0.01$). In addition, IGF1 significantly ($P<0.05$) increased GH3 cell viability by 20–60% at concentrations ranging from 10 to 500 nM, independently of the concentration. These proliferative effects were completely abrogated by co-incubation with 50–100 nM Everolimus or NVP-BEZ235. On the other hand, 50–100 nM Everolimus or NVP-BEZ235 significantly promoted caspase 3/7 activity (15–25%; $P<0.02$. Basal apoptotic rate was not significantly influenced by IGF1, which did not protect GH3 cells from proapoptotic effects of Everolimus and of NVP-BEZ235. These results confirm that IGF1 has proliferative effects on pituitary adenoma cells, which are mediated, at least in part by mTOR. On the contrary, IGF1 does not prevent the pro-apoptotic effects of mTOR inhibitors.

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P830

Mutations of SOX2 gene: a novel heterozygous mutation and impact on congenital hypopituitarism

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Introduction
Anophthalmia/microphthalmia is a rare developmental craniofacial defect often associated to congenital hypopituitarism with GH deficiency and hypogonadism. SOX2 gene plays a key role in embryonic development regulation and heterozygous mutations of this gene, reported only in 14 patients to date, have been associated to anophthalmia/microphthalmia and congenital hypopituitarism in uo to 10% of cases. Therefore, the study of the SOX2 gene can be clinically useful in determining etiology and appropriate therapeutic approaches.
Patient and methods
We report the case of a novel SOX2 gene mutation in a 17 years old male patient with congenital anophthalmia and hypopituitarism. Brain magnetic resonance imaging (MRI) showed complete agenesis of corpus callosum and ocular bulbs with optic nerve hypoplasia, whereas hormonal assays revealed GH deficiency and hypogonadotropic hypogonadism requiring the appropriate replacement therapy. Familial investigation did not show the same phenotype in any of first-degree relatives. The coding region of the SOX2 gene was sequenced.
Results
The novel heterozygous SOX2 inactivating mutation c.905delC, predicting the frameshift p.Pro302Arg fs*69, was found. Genetic counseling was recommended in all first-degree relatives.
Conclusions
This case illustrates the importance of genetic testing of SOX2 gene to aid correct diagnosis and to assist in clinical management.

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P831

Chracterizazion of somatostatin receptor expression in MENX-assoxciated pituitary adenomas: impact on therapy and imaging with somatostatin analogs

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Introduction
Somatostatin analogs (SSAs) are the first-line clinical treatment for patients with pituitary adenomas (PAs) but their efficacy is highly variable among patients. This could be due to a differential expression of somatostatin receptor subtypes (Ssts) among tumors, but this issue is still unresolved. Nonfunctioning pituitary adenomas (NFPAs), which mostly derive from gonadotroph cells, are among the tumors that poorly respond to SSAs. Rats carrying a germline loss-of-function mutation in p27 (MENX syndrome) develop gondotroph adenomas that closely resemble human gonadotroph tumors. To determine whether rat PAs can be used to explore the relationship between Sst expression and response to SSA, we determined the expression profile of Ssts in these tumors and assessed their response *in vitro* to the SSA octreotide.
Methods
Adenoma tissues or primary PA cells from MENX-affected rats were used. qRT-PCR, immunohistochemistry (IHC) and *in vitro* autoradiography were performed to determine Ssts expression. Rat primary PA cells were treated with the octreotide and cell viability assessed. Small animal positron emission tomography (PET) was performed using the somatostatin agonist 68Ga-DOTA-NOC.
Results
We observed higher expression of *Sst2* and *Sst3* mRNA in PA cells from mutant rats when compared with cells from wild-type rats. Accordingly, Sst2 protein was found more highly expressed in the rat tumors (especially the small ones) than in non-tumorous pituitary areas by IHC. Sst3 protein was also detectable in the rat tumors. Treatment of rat primary PA cells *in vitro* with octreotide showed that they partially respond to the drug, similarly to human NFPAs. By 68Ga-DOTA-NOC-PET imaging, we found the increased uptake in the pituitaries of mutant rats *in vivo* when compared with wild-type rats.
Conclusion
Studies of MENX-associated PAs might help us understand the relationship between Sst expression and response to SSAs in NFPA.

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P832

Clinical and morphological characteristics of dopamine agonist-resistant prolactinomas

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Introduction
The aim of our study was to investigate clinical and morphological features of dopamine agonist-resistant prolactinomas.
Methods/design
The study included 51 patients (38 women, 13 men) with prolactin-secreting pituitary adenoma that is resistant to treatment with dopamine agonists, age 15–82 years.
Results
Median of the serum PRL level distribution at the onset of the disease was 4500 mIU/l (3480, 140 300). The MRI showed nine patients (17.6%) had microadenoma, 42 patients (82.4%) – macroadenoma, in 14.3% of the cases a giant adenoma was found. 31 patients underwent surgery (60.8%), and six patients were operated repeatedly. Radical surgical treatment, estimated as reaching normoprolactinemia and no residual adenomatous tissue, was seen in 35.5% of the cases (11 patients). In addition, five patients (16.1%) the surgery resulted in medical control of hyperprolactinemia with lower doses of cabergoline than those before the surgery. 20 adenoma samples were investigated by immunohistochemistry. In the study of the expression of PRL the diagnosis of prolactinoma was confirmed in 19 patients (95%), one patient did not reveal any expression of tropic hormones. Co-expression of the GH was found in seven patients (36.8% of the 19 patients with PRL immunopositivity), although clinical acromegaly was not observed in these patients, with normal values of IGF1. The expression of TSH, ACTH, LH, FSH was not found in any case. The expression of dopamine receptor type 2 was confirmed in 13 cases (65%). The presence of somatostatin receptor expression type 2 (SSR2) was detected in five patients (25%), SSR5 – in eight patients (40%), SSR2 and SSR5 – in three patients. Estrogen receptor expression was detected only in one case (5%). None of the patients showed expression of progesterone receptors.
Conclusion
Resistance to treatment with dopamine agonists is a serious problem; more often patients with resistant prolactinomas have macroadenoma. Surgical treatment is effective in 35.5% of cases. We can assume that resistance to treatment with dopamine agonists is associated with low expression of dopamine receptors in the

cells of removed adenomas. Low expression of SSR2 and SSR5 in these patients may indicate the failure of therapy with somatostatin analogues. Thus, further study and search for effective treatments for these patients are required.

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P833

Expression of connexins 26, 32 and 43 mRNA in normal and pituitary adenomas

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Connexins are a proteins critically involved in the formation of gap junction, which connect cells with each other and enable the exchange of small compounds and, besides having a role independently of gap junction formation. At least, in the anterior pituitary two cellular networks have already been identified, one of them is composed of the endocrine inactive folliculo-stellate cells and the second one is the GH-producing somatotroph cell network. It is supposed that gap junctions may play an important role in these networks. The network organization of endocrine and non-endocrine cells of the anterior pituitary may be of high functional relevance and may allow a more rapid and coordinated release of hormones and/or growth factors. Loss of these network structures during non-coordinated pituitary adenoma cell growth may play a role in the formation of pituitary tumors, as it has already been reported from other forms of solid tumors, in which disturbed connexins expression has been observing. The aim of this work is to determined mRNA expression of connexin 26, 32 and 43 in normal pituitary and endocrine-inactive adenomas, somatotropinomas and corticotropinomas. We determined mRNA connexin expression using real time reverse transcriptase PCR (qRT-PCR) in series of pituitary tumors. Our initial results demonstrated a decrease in connexins expression in most of adenomas studied. We observed a decrease an expression in connexin 26 in 12 tumors of 14 non-functioning adenomas, in six tumours of seven corticotropinomas and in four tumours of four somatotropinomas when compared it with normal pituitary connexin 26, 32 and 43 expression. We analyzed connexin 32 mRNA expression in these adenomas the data showed that a decrease in connexin it expression in ten adenomas of 13 non-functioning adenomas, in five adenomas of five corticotropinomas and in five adenomas of five somatotropinomas. Finally, we also observed a decrease in connexin 43 mRNA expression in 12 adenomas of 14 non-functioning adenomas, in three adenomas of five corticotropinomas and in five adenomas of five somatotropinomas. Taken together, these data raise questions about the possibility of connexins are participating in the tumorigenesis and/or the pathophysiology of pituitary adenomas.

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P834

No correlation between somatostatine 2 receptor expression analyzed by RNA *in-situ* hybridization and real-time qRT-PCR in clinically non-functioning pituitary adenomas

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Objective
The aim of this study was to quantitatively estimate SSTR2 in clinically non-functioning pituitary adenomas (CNFAs) with use of RNA *in-situ* hybridization (ISH) and quantitative real-time RT-PCR and correlate the results of both methods.
Methods
A standard histological and immunohistochemical examination was performed on the resected pituitary tumour. Afterwards *in-situ* hybridization for somatostatine 2 receptor (SSTR2) RNA was performed manually using the RNAscope® 2.0 FFPE Assay (Advanced Cell Diagnostics, Inc., Hayward, CA, USA) on formalin fixed and paraffin embedded tissue sections. Small part of the same tumour resected during operation and stored in RNAlater was used for quantitative real-time RT-PCR.
Results
A 25 adenomas with SSTR2 mRNA expression in qRT-PCR were chosen for further evaluation with RNA ISH. SSTR2 mRNA was expressed in all adenomas from 1413–148 680 copies/5 µl cDNA; the median of relative quantity (after normalization to housekeeping gene GUS) for SSTR2 was 111%. In contrast to qRT-PCR immunostaining was positive only in nine adenomas with the use of RNA ISH. Positive cases were subsequently semi-quantitatively assessed according to the manufacturer's scoring guideline as follows: 1 adenoma with 1+, 5 with 2+ and 3 with 3+. No adenoma scored 4+ although high expression of SSTR2 mRNA was present. We did not find any correlation between data (Spearman's rank correlation coefficient 0.243).
Conclusion
Use of somatostatine analogues or dopastatins remains controversial in CNFAs. Verification of SSTR presence before use of drug treatment should be useful. Both RNA ISH and qRT-PCR have their pitfalls. In our case, we did show no correlation between these methods. RNA ISH is definitely less sensitive and specific. We think that interpretation of results for SSTR2 expression in RNA ISH and qRT-PCR should be very careful.
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P835

FSH and LH cells in normal-fed male rats after centrally applied ghrelin

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Ghrelin, the endogenous ligand of GH secretagogue receptor type 1a, has emerged as pleiotropic modulator of diverse biological functions, including energy homeostasis and recently, reproduction. The effects of i.c.v. administered ghrelin on morphological characteristics of pituitary FSH- and LH-cells were examined in adult male Wistar rats. The animals were randomized in two groups: control and experimental group, each consisted of seven animals, and implanted with an i.c.v. cannula. After a recovery period, experimental males received 1 µg ghrelin/5 µl PBS during 5 days, while control males received the same volume of saline vehicle. Experimental and control males were sacrificed under ether narcosis 2 h after the last injection. FSH- and LH-producing cells were studied using the peroxidase–antiperoxidase (PAP) immunohistochemical procedure. The FSH and LH cells of adult control males were polygonal, oval or polyhedral in shape with prominent, often eccentrically located nuclei. They were strongly immunohistochemically stained and positioned throughout the pituitary pars distalis, alone or in groups, often in close contact with blood capillaries. After i.c.v. treatment with ghrelin, FSH and LH cells in the pituitaries were smaller in size, less intensely immunostained and their shape was irregular comparing to the controls.
In animals i.c.v. treated with ghrelin volume of FSH cells and volume of their nuclei were significantly decreased ($P<0.05$) by 34.0 and 37.1% respectively, while these parameters of LH cells were unchanged when compared to the control values. The volume density of LH cells in animals i.c.v. treated with ghrelin was significantly decreased ($P<0.05$) by 38.9%, but the volume density of FSH cells was not altered in comparison with the controls. In conclusion, our results indicate that i.c.v. applied ghrelin have inhibitory effect on the volume of FSH cell and volume of their nuclei, as well as on relative volume density of LH cells.

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P836

PTTG and Ki-67 expression in pituitary adenomas

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Introduction
During clinical observation the behaviour of pituitary tumours is often unpredictable. The work was aimed at evaluating Ki-67 and pituitary tumour transforming gene (PTTG) indices in pituitary adenomas.
Material and methods
The Ki-67 and PTTG indices were determined by immunochemistry in specimens excised from neurosurgically removed pituitary tumours. In fifty two examined patients (30 females and 22 males, mean age 50.5 ± 15.4 years) who underwent pituitary tumour surgery, micro- and macroadenoma occurred in 10 and 42 cases respectively.
Results
The expression of Ki-67 and PTTG were revealed only in cells of pituitary tumours (absent in extra-tumoural tissue) and was present in 79 and 83% of adenomas respectively. The median values of Ki-67 and PTTG indices were 1.56% (IQR 3.05) and 1.02% (IQR 2.27) respectively. The index of PTTG, contrary to Ki-67 index, was significantly higher in adenomas with positive anterior pituitary hormone expression ($n=40$) as compared with adenomas with negative anterior pituitary hormone expression ($n=12$) (1.22 (IQR 2.17) vs 0.12 (IQR 1.07), $P<0.05$). Both Ki-67 and PTTG indices were not significantly related to tumour size category (microadenoma/macroadenoma) and were not correlated with tumour size. Contrary to Ki-67, PTTG index was significantly correlated with patient age ($r=-0.267$, $P=0.026$). We found no correlation of both above-mentioned indices with expression of pituitary hormones in examined specimens.
Conclusion
Expression of Ki-67 and PTTG was observed in the majority of pituitary adenomas. Ki-67 and PTTG expression, as determined in pituitary tumour specimens, was not related to the tumour size and kind of pituitary hormone expression.

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P837

Medical therapy of acromegaly

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Introduction
Medical therapy of acromegaly is indicated in patients who failed to achieve remission after surgery, with contraindication or refusal for surgical therapy or following radiotherapy in order to bridge the interval until complete remission.
Objective
Evaluate the medical therapy – somatostatin analogs (SSA), dopamine agonist (DAs) and GH receptor-antagonist (Pegvisomant) – in acromegalic patients followed from 1988–2011.
Methods
We conducted a retrospective study of 52 acromegalic patients (32 women) submitted to medical therapy.
Criteria for disease control (normal age- and sex-adjusted IGF1 concentration and GH below 1 ng/ml) were evaluated by the average of three values. A reduction below 20% of those parameters after SSA was considered as an absence of response to this therapy.
Results
Medical treatment was the only option for eight patients, complemented surgery in 29, isolated radiotherapy in one, and radiotherapy combined with surgery in 14. SSA were used in 43 patients (seven experienced no response). A normal GH and IGF1 concentration was achieved in 16.2% of 36 patients, with an average IGF1 reduction of 54.2%.
DAs were added in 12 patients insufficiently responding to SSA monotherapy, with an IGF1 reduction of 10.7%. Only one case showed normalization of GH and IGF1 concentration.
Six patients on SSA plus DAs *ab initio* achieved IGF1 reduction of 66.8%, with only one case coming under control. Pegvisomant enabled the control of acromegaly in four of seven cases, with an IGF1 reduction of 48.7%
In total, 26.5% ($n=13$) of the patients were under control. Of those not controlled ($n=36$), 50% displayed a dissociated response (IGF1 or GH) and 40.6% registered IGF1 levels only 10% above upper limit of normal.
Conclusion
Around 25% of our patients were controlled. Half of patients not under control displayed a dissociated response. Adding DA to SSA doesn't seem to improve the results.

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P838

Daidzein affects the stereological parameters of pituitary GH cell in orchidectomized adult rat

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Daidzein, one of the main phytoestrogens of soy, is able to act as estrogen-like compounds due to its structural similarity with 17β-estradiol, which gives them the capacity to bind estrogen receptors and to induce hormone-like effects. Considering, that somatotropic system is sensitive to sex steroids, the aim of this study was to investigate the effects of daidzein on stereological and biochemical parameters of pituitary somatotropic (GH) cells in orchidectomized adult rat. Adult Wistar rat were divided into two groups: orchidectomized rat s.c. treated with medium (absolute etanol and sterile olive oil mixture; Orx) and orchidectomized rats s.c. treated with daidzein in medium (30 mg/kg BW; Orx + D). The groups of animals received the treatment during three weeks and were sacrificed 24 h after the last injected dose. Immunohisochemically labeled pituitary section were stereologically analysed, using NewCast Stereological Software Package. The circulating GH was determined biochemically. The volume density of pituitary GH cells was increase ($P<0.05$) after daidzein treatment ($25.6 \pm 2.4\%$), in comparation to Orx group ($20.1 \pm 1.15\%$). In addition, intensity of staining of GH cell and volume of GH cells were increased ($P<0.05$) after daidzein treatment ($2172.6 \pm 110.8\ \mu m^3$), in comparision with Orx group ($1203.8 \pm 174.9\ \mu m^3$) as well. Numerical density and absolute number of GH cells were decrease ($P<0.05$) after daidzein treatment ($10.8 \pm 0.6 \times 10^4\ mm^{-3}$; $5.6 \pm 0.8 \times 10^5$ respectively), in comparison to Orx group ($16.8 \pm 2.1 \times 10^4\ mm^{-3}$; $7.6 \pm 0.9 \times 10^5$). The treatment did not affect GH level in blood. These results indicated that daidzein has provoked diferent changes of the stereological parameters of GH cells, which did not affect GH blood level. It can be concluded that response of somatotropic system to daidzein treatment includes, beside regulation at the level of the pituitary gland, other mechanisms of regulation of GH secretion.

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P839

8-Prenylnaringenin decreases hormone expression in GH3 pituitary adenoma cells of the rat

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Objective
8-Prenylnaringenin (8-PN) is a phytoestrogen discovered in hops. GH3 pituitary adenoma cells of the rat show a variety of responses to phytoestrogens *in vitro*. This *in vitro* study aims to analyze changes in hormone expression in GH3 cells treated with 8-PN.

Methods
GH3 cells were incubated with 0.5–50 μM 8-PN for 4 h. Medium and ethanol served as controls. Changes in expression of growth hormone (GH) and prolactin (PRL) normalized to β2 microglobuline (β2MG) were assessed using quantitative real time PCR (QRT-PCR). T served as statistical test. $P<0.05$ was supposed to be statistically significant.
Results
In GH3 cells treated with 0.5–50 μM 8-PN, expression of GH and PRL is decreased as compared to controls. Significant differences in GH expression were found after treatment with 0.5 μM 8-PN as compared to ethanol ($P=0.042$), 0.5 μM 8-PN as compared to medium ($P=0.011$), 5 μM 8-PN as compared to medium ($P=0.022$) and 50 μM 8-PN as compared to medium ($P=0.013$). Significant differences in PRL expression were found after treatment with 0.5 μM as compared to ethanol ($P=0.039$) and 50 μM 8-PN as compared to ethanol ($P=0.046$).
Conclusion
Further investigation of the impact of 8-PN on hormone expression and secretion in GH3 cells is warranted.

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Pituitary – Clinical (*Generously supported by IPSEN*)

P840

Early and sustained tumour volume reduction and GH/IGF1 control in patients with GH-secreting pituitary macroadenoma primarily treated with lanreotide Autogel 120 mg for 48 weeks: the PRIMARYS study

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Introduction
First-line somatostatin analogue treatment may be an effective alternative option to surgery for some patients with GH-secreting pituitary macroadenoma. The PRIMARYS study aimed to investigate the impact of primary lanreotide Autogel 120 mg treatment on tumour volume and GH/IGF1 control in treatment-naïve acromegalic patients over a one-year time course.
Methods
PRIMARYS was an international, multicentre, open-label, single arm, phase 3b study (NCT00690898/EudraCT2007-000155-34). Treatment-naïve acromegalic patients with GH-secreting pituitary macroadenoma received primary therapy with lanreotide Autogel 120 mg every 28 days for 48 weeks (12 injections). The primary endpoint was percentage of patients with ≥20% reduction in tumour volume from baseline to week 48 based on MRI central assessments from three readers. The primary analysis used the reader with the best standardized sensitivity determined on repeatability tests. Tumour volume and hormonal status were assessed at 3, 6, and 12 months after therapy initiation. Efficacy was assessed in the intention-to-treat population.
Results
Ninety patients received treatment (baseline mean maximum adenoma diameter 19.0 mm, tumour volume 2739 mm3, GH 15.0 μg/l, IGF1 810 μg/l). The primary analysis showed 56/89 (63%) patients achieved ≥20% reduction in tumour size (95% CI: 52–73%). During the 48 weeks' treatment, there was an early and sustained reduction in tumour volume over time (mean change: W12, −20%; W24, −25%; W48, −27%); this was mirrored by improvements in GH (W12, −62%; W24, −65%; W48, −71%) and IGF1 levels (W12, −44%; W24, −47%; W48, −57%; Figure). Overall, lanreotide Autogel 120 mg was well tolerated throughout the study period.
Conclusions
This large prospective study enrolling treatment-naïve acromegalic patients with GH-secreting pituitary macroadenoma demonstrated that primary treatment with lanreotide Autogel 120 mg every 28 days rapidly achieved tumour reduction of pituitary adenoma volume and sustained GH/IGF1 control. These data support further exploring the potential use of lanreotide as an initial therapeutic option in this patient population.

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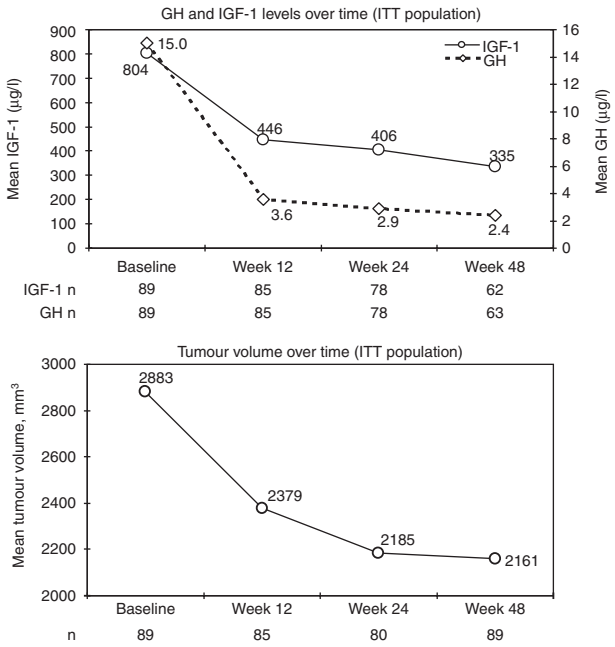

P841

The benefits of pasireotide in patients with Cushing's disease are not restricted to patients with normalisation of UFC; results from a large, 12-month study

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Introduction
Pasireotide normalized or reduced UFC in patients with Cushing's disease in a large, 12-month study. This analysis evaluates the effects of pasireotide on the signs/symptoms of Cushing's disease according to the degree of UFC control.
Methods
Adult patients ($n=162$) with persistent/recurrent or *de novo* Cushing's disease were randomized to pasireotide 600/900 μg s.c. bid. Dose titration (max: 1200 μg bid) was allowed after month 3 and initiation/change in antihypertensive, antidiabetic and lipid-lowering medications was permitted throughout the study. Changes in the symptoms of Cushing's disease during pasireotide treatment were evaluated at 6 and 12 months. UFC measurements were conducted at central laboratories. UFC control was defined as UFC ≤ ULN, partial control as UFC > ULN but with at least 50% reduction from baseline, and uncontrolled UFC as UFC > ULN without a ≥50% reduction from baseline.
Results
In general, improvements in blood pressure, weight and BMI were observed in patients with and without UFC control, although the greatest changes were observed in those with UFC control (see Table). Similar results were seen for facial rubor and striae. Fasting plasma glucose and HbA1c levels increased from baseline in all patients receiving pasireotide, irrespective of UFC control. The relative impact of concomitant medications on signs and symptoms could not be evaluated.

Table 1

	Change from baseline to month 6 (mean (95% CI))			Change from baseline to month 12 (mean (95% CI))		
	C ($n=32$)	PC ($n=22$)	U ($n=62$)	C ($n=28$)	PC ($n=17$)	U ($n=33$)
SBP (mmHg)	−13.4 (−20.1, −6.8)	−7.5 (−15.5, 0.4)	−7.3 (−11.4, −3.2)	−11.8 (−18.0, −5.6)	−3.8 (−11.6, 4.1)	−2.5 (−8.0, 3.1)
DBP (mmHg)	−7.7 (−12.3, −3.2)	−3.9 (−9.8, 2.0)	−3.2 (−6.1, −0.3)	−7.3 (−11.4, −3.2)	−3.2 (−8.7, 2.3)	−0.9 (−4.6, 2.8)
BMI (kg/m^2)	−2.1 (−2.7, −1.5)	−1.2 (−2.1, −0.4)	−1.5 (−1.9, −1.1)	−2.9 (−3.8, −2.1)	−1.6 (2.6, −0.6)	−2.5 (−3.3, −1.8)
Weight (kg)	−5.6 (−7.2, −4.0)	−3.2 (−5.3, −1.1)	−4.1 (−5.2, −3.1)	−8.0 (−10.3. −5.6)	−4.2 (−6.8, −1.6)	−6.9 (−8.9, −4.9)

C, controlled; PC, partially controlled; U, uncontrolled; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Conclusions
Reduction in UFC levels observed during treatment with pasireotide was accompanied by corresponding improvements in the signs and symptoms of Cushing's disease, which were maintained for 12 months. Importantly, improvements were observed even without complete UFC normalization, suggesting that partial improvements in UFC produced by pasireotide may be beneficial in improving signs and symptoms in patients with Cushing's disease.

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P842

Exploration of hand size as a screening tool for acromegaly

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Introduction
There is a long delay between the onset of symptoms and correct diagnosis of acromegaly. This study aimed to explore the potential of hand size as a screening tool of acromegaly. Here we present first results of a new screening project.
Method
We measured scan-copies of acromegalics ($n=27$, 9 women, age 18–84 years) and controls ($n=111$, 71 women, age 19–81 years) and analyzed surface areas of palm and thenar and maximal diameters of hand, thenar and proximal fingers.
Results
The difference of palm surface areas and diameters between acromegalics and controls is highly significant. Most in men, where palm surface areas of acromegalics to controls (192.22 ± 15.24 vs 167.93 ± 16.37 cm, $P<0.001$) and hand diameters (9.61 ± 0.50 vs 8.87 ± 0.46 cm, $P<0.001$) reached same significance. In women, palm surface areas (145.02 ± 10.87 vs 133.90 ± 11.30 cm, $P<0.01$) and hand diameters (8.24 ± 0.32 vs 7.84 ± 0.39 cm, $P<0.005$) of acromegalics were significant higher compared to controls.
In controls, the palm surface area at the 95th percentile (representing a specifity of 95%) was 192.07 and 152.90 cm^2 and the hand diameter at the 95th percentile was 9.58 and 8.39 cm in men and women respectively. The corresponding sensitivities to these cutoff levels were 55 and 33% for palm surface area and 55 and 44% for hand diameter in men and women respectively.
Conclusions
Measures of hand size may be a helpful and simple tool in the early screening for acromegaly. This approach allows defining clinical cutoff levels with a high specifity, however at a cost of low sensitivity. Further measurements with a higher sample size will follow for more robust results.
Declaration of funding
This study was partially supported by Pfizer.

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P843

The results of sandostatin LAR therapy in acromegalic patients

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Introduction
Somatostatin analogs, formerly used as adjuvants in acromegaly treatment, are widely used as primary treatment today.
Objective
To investigate the efficacy of octreotide therapy in acromegalic patients as primary or secondary therapy.
Materials and methods
Four acromegalic patients diagnosed at the Endocrinological Clinic (males, mean age 35–75 years, age range 26–49 years, all patients with macroadenoma) were treated with octreotide. Among them, three patients received octreotide preoperatory and one postoperatory. The concentration of human GH (HGH) and IGF1 was evaluated at 0, 6 and 12 months, while magnetic resonance imaging (MRI) was taken before treatment and 12 months after. All pacients received initially octreotide 20 mg/28 days. The dose was adjusted by the individual patient's response. Mean treatment duration was 25–75 months (range 6–43 months).
Results
Mean serum GH fell from 53 ng/ml (range 51.7–130.6) to 13 ng/ml (range 2.03–29.83) at the 3 months visit and remained suppressed. Mean plasma IGF1 concentrations fell from 502.5 ng/ml (range 327–838) to 490.25 ng/ml (range 320–677) at three months. Patients with higher initial GH concentrations were less likely to normalize IGF1 concentrations during treatment. After 1-year of therapy, tumor size decreased with a mean value of 22.58% in 75% of cases. Dose increments above 30 mg/28 days at one patient did not provide additional benefit in terms of hormonal reduction. The symptoms were ameliorated in 75% of patients. The most common adverse events were gastrointestinaly; one patient developed gallstones but was asymptomatic.
Conclusions
Octreotide treatment of acromegaly not only decreases GH and IGF1 concentrations, but also appears to diminish the size of the tumor.

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P844

Spanish molecular registry of pituitary adenomas: a multicenter, translational approach aimed at improving patient management

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Pituitary adenomas are heterogeneous, rare tumors, which hinders analysis of large numbers of cases with common approaches. To overcome this, a multicenter, clinical-basic strategy was proposed aimed at enhancing the tools

to diagnose and manage pituitary tumors by combining clinical/pathological/molecular information. This initiative was developed by the Sociedad Andaluza de Endocrinología y Nutrición, and further endorsed by the Sociedad Española de Endocrinología y Nutrición, and supported by Novartis. A comprehensive strategy based on a coordinated network was designed covering all Spanish reference centers for pituitary pathology. To develop a shared database registering clinical, pathological and molecular information for each patient and minimize intercenter variability, common protocols and methods were set up for tissue collection, clinical, pathological and molecular data analysis and registry. This joint initiative, named Spanish molecular registry of pituitary adenomas (REMAH) was established in 2010 and organized in six regional nodes. A common database has been generated (http://www.remahnacional.com) including clinical and pathological information. A standardized system for adenoma molecular phenotyping was developed and validated. Specifically, 26 genes were evaluated by quantitative real-time PCR, including pituitary hormones, receptors for somatostatin, dopamine, GHRH, GnRH, CRH, vasopressin and ghrelin, plus additional selected markers (Ki-67, PTTG-1) and three housekeeping genes for normalization (β actin, HPRT, and GAPDH). Molecular information was obtained on 250 tumors, out of 634 patients registered until 2012. Initial analysis indicates a close parallelism of the molecular profile of the adenoma subtypes with previously reported data and with the clinical phenotype of the patients, and also provide additional information on specific receptors known as drug-targets. REMAH is a unique, country-wise, multi-centric, multi-disciplinary network of expertise supported on a shared database enabling a translational, more powerful approach to the management of pituitary adenomas, paving the way for innovative clinical-basic studies with large numbers of patients of these rare pathologies.

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P845

Pasireotide LAR and octreotide LAR maintain inhibition of GH and IGF1 in patients with acromegaly: 12-month extension phase of a randomized, double-blind, multicenter, phase III study

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Introduction

Pasireotide LAR was significantly superior to octreotide LAR at providing biochemical control in a 12-month trial in 358 medically naïve patients with acromegaly. Patients with clinical benefit or GH <2.5 μg/l and IGF1 $\leq$ ULN could continue therapy in the extension study.

Methods

Patients entering the extension (pasireotide LAR, $n=74$; octreotide LAR, $n=46$) were followed up to month 26 (core plus extension) for octreotide LAR, whereas pasireotide LAR patients could continue beyond month 26. Dose titration to pasireotide LAR 60 mg/28 days or octreotide LAR 30 mg/28 days (if GH ≥ 2.5 μg/l and/or IGF1 $>$ ULN) or to pasireotide LAR 20 mg/28 days or octreotide LAR 10 mg/28 days (for tolerability) was permitted throughout core and extension.

Results

Mean duration of exposure was 465 days (pasireotide LAR) and 412 days (octreotide LAR). 51 pasireotide LAR and 36 octreotide LAR patients completed month 26. Suppression of GH and IGF1 was maintained throughout the extension in both arms. Median GH (μg/l) and IGF1 (×ULN) in pasireotide LAR vs octreotide LAR patients were: at baseline, 8.8 vs 10.1 and 2.9 vs 2.9; at month 12, 1.9 vs 2.0 and 0.9 vs 1.3; and at month 25, 1.0 vs 1.0 and 0.6 vs 0.9. Median percentage change in GH and IGF1 at month 25 was -83% and -71% with pasireotide LAR and -86% and -64% with octreotide LAR. Mean percentage change in tumor volume from core baseline to month 25 was -51.8% ($n=54$) for pasireotide LAR and -55.0% ($n=34$) for octreotide LAR. Both treatments improved acromegaly symptom scores. Most common AEs during the core and extension in both treatment arms were diarrhea, cholelithiasis, hyperglycemia, headache, and diabetes mellitus. Hyperglycemia-related AEs were more frequent with pasireotide LAR than octreotide LAR (62.9% ($n=112/178$) vs 25.0% ($n=45/180$)).

Conclusions

These results suggest that pasireotide LAR and octreotide LAR provide long-term inhibition of GH and IGF1 in patients with acromegaly.

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P846

Characteristics of patients with pituitary gigantism: results of an international study

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Aim

To analyse a large series of patients with pituitary gigantism.

Materials and methods

We included in this multicentre study 158 patients (129 males) with pituitary adenoma (PA) or hyperplasia and current/previous abnormal, excessively rapid growth velocity for age or a final height greater than 2 SD above normal for their population. Data of patients were systematically recorded in case report forms.

Results

The first symptoms developed at median age of 15 years (11–19). 96% had facial changes and/or acral overgrowth at time of diagnosis. Age at diagnosis: PA in females were younger than in males (16.5 vs 23 years) with median delay in diagnosis of PA of 5 years. Twenty-four patients were still growing and had median height of 191 cm (171–199). One hundred and thirty-four patients had stopped growing at age of 20 years (18–22). The relative difference from calculated midparental height was significantly greater in those with control of GH-excess after 20 years old than before (10.9% (8.4–15) vs 7.9% (5.9–10.3), $P=0.012$). Most PA were macroadenomas (84%), with median maximal tumor size of 25 mm (14.5–37). More than 50% of cases had extrasellar extension (77%) or invasion (54%). 4% had pituitary hyperplasia. One hundred and forty-five patients were operated with remission after first operation in 14% and in 0% in those who were reoperated (26 patients). Multimodal treatment approach was in 40% and disease control was achieved in 43% (median follow up on treatment was 7 years (2–16)). Hypopituitarism increased in frequency from 24% at

baseline to 69% at last follow-up. Genetic/inherited features were seen in 34% at presentation and included syndromes like FIPA (with and without AIPmut), McCune-Albright, Carney complex, one case of familial pituitary hyperplasia. Overall, germline mutations in AIP gene were found in 43.6% (24/55) of those tested.

Conclusion

Patients with pituitary gigantism are predominantly male. Somatotropinomas in patients with gigantism are mostly large (with extrasellar extension and invasion in more than half of cases) and difficult to control. Treatment delay may increase the harm from GH-excess, particularly on tall stature. Syndromic features are presented in 1/3 of cases and AIP mutations are common in the tested population.

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P847

Switching patients with acromegaly from octreotide LAR to pasireotide LAR improves biochemical control: crossover extension to a randomized, double-blind, multicenter, Phase III study

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Introduction

In a Phase III trial, pasireotide LAR was significantly superior ($P=0.007$) to octreotide LAR at providing biochemical control at 12 months in medically naïve acromegaly patients (post-pituitary surgery or *de novo*). Inadequately controlled patients (GH $\geq$ 2.5 μg/l and/or IGF-1 > ULN) at the end of core study were eligible for switching therapy (crossover extension). Reported here are efficacy results up to 12 months and safety results up to 13 months post-crossover.

Methods

Eligible patients were switched to either pasireotide LAR 40 mg/28 days ($n=81$) or octreotide LAR 20 mg/28 days ($n=38$). One dose escalation to pasireotide LAR 60 mg/28 days or octreotide LAR 30 mg/28 days was permitted, but not mandatory, post-crossover. Main outcome measures included GH < 2.5 μg/l and normal IGF-1 12 months after switching medical therapy.

Results

A 31 pasireotide LAR and 13 octreotide LAR patients discontinued within 12 months after crossover. Response rates (95% CI) 12 months after crossover to pasireotide LAR ($n=81$) and octreotide LAR ($n=38$), respectively: GH < 2.5 μg/l and normal IGF-1, 17.3% (9.8–27.3) and 0%; normal IGF-1, 27.2% (17.9–38.2) and 5.3% (0.6–17.7); GH < 2.5 μg/l, 44.4% (33.4–55.9) and 23.7% (11.4–40.2); tumor volume decreased from extension baseline by a mean (SD) of 24.7% (25.2) and 17.9% (27.8). A significant (≥20%) tumor volume reduction from extension baseline was seen in 54.3% (25/46) of pasireotide LAR and 42.3% (11/26) of octreotide LAR patients. Safety profile of both agents was consistent with that seen in the core trial and with somatostatin analogues. Hyperglycemia-related AEs, while mostly mild or moderate, were more frequent with pasireotide LAR (64.2%) than octreotide LAR (21.1%). Fasting plasma glucose and HbA_{1c} levels decreased to near normal levels within 3 months after switching from pasireotide LAR to octreotide LAR.

Conclusions

Pasireotide LAR holds promise as a treatment option for acromegaly patients inadequately controlled with octreotide LAR. Hyperglycemia associated with pasireotide LAR appeared to be reversible upon discontinuation of pasireotide.

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P848

Advanced glycation end product associated skin autofluorescence and serum corboxymethyl lysine levels in acromegaly

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Aim

Nonenzymatic advanced glycation and oxidation end-products, advanced glycation end-products (AGEs), impart a potent impact on vessels in diabetic state and in euglycaemic conditions with increased oxidative stress.
The aim of this study is to measure AGEs and its relationship with carotid intimae media thickness (CIMT) in acromegaly patients.

Method

A case–control study was performed in 225 acromegalic patients (F/M: 116/109, 50.6 ± 11 years.) and age and sex matched 100 controls (F/M: 58/42, 52.6 ± 11 years.).Skin autofluorescence (SAF) is a validated noninvasive measure of tissue AGEs SAF was measured with the AGE Reader. Serum carboxymethyl lysine (CML) was measured with ELIZA method. HbA1c and growth hormone was measured by HPLC and immunochemiluminescence method respectively. CIMT was assessed with Doppler ultrasonography.

Results

SAF was higher inacromegalic patients (1.95 ± 0.32 arbitrary units (AU) compared with controls (1.62 ± 033 AU) ($P=0.003$). serum CML levels were higher in acromegalics (0.245 ± 0.11 ng/dl) compared with controls (0.175 ± 0.07 ng/dl) ($P=0.002$). CIMT measures were 0.62 ± 0.14 and 0.59 ± 0.14 mm for acromegalic and control groups respectively ($P<0.0001$). Correlation analysis showed a positive correlation between SAF and serum CML ($r=-0.35$, $P=0.002$), CIMT ($r=0.25$, $P=0.004$) and serum growth hormone levels ($r=0.33$, $P=0.03$). sCML levels were higher in uncontrolled acromegalics compared to uncontrolled patients. Accumulation of tissue AGEs is increased in acromegalic patients.

Table 1 SAF, sCML and A1c levels in diabetic and non diabeticacromegalic patients

	Diabetes (+) acromegalic patients	Diabetes (−) acromegalic patients	Control group	*P*
A1c(%)	7.05 ± 1.1	5.4 ± 0.6	5.4 ± 0.4	0.001
SAF (AU)	1.93 ± 0.3	1.88 ± 0.6	1.62 ± 0.37	0.01
sCML (ng/dl)	0.245 ± 0.11	0.172 ± 0.06	0.174 ± 0.12	0.02

Conclusion

SAF and sCML levels increased in acromgalic patients regardless of hyperglycemia. AGE may have a role in the cardiovascular outcomes of acromegalic patients

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P849

The prevalence of insufficient test responses in patients with traumatic brain injury compared to healthy controls – results from The Danish National Study on posttraumatic hypopituitarism

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Introduction

We questioned the justification of general recommendations for assessment of hypopituitarism in patients with traumatic brain injury (TBI), and aimed to describe the prevalence of hypopituitarism in a national TBI population of patients admitted to a Danish hospital in 2008, as compared to healthy controls.

Patients and methods

We included 463 patients (18–65 years) hospitalized ≥24 h, with more than subtle TBI as indicated by loss of consciousness, amnesia, or cranial/cerebral imaging abnormalities. The patients underwent endocrine assessment median 2.5

years (range 1.0–4.4) post TBI. Assessment included baseline evaluation of thyroid and gonadal hormone concentrations, and dynamic assessment of the GH and HPA axis. Results were compared to those from healthy controls. Deficiencies were defined according to local assay and test specific cut-offs.
Results
An insufficient 30 min cortisol response to Synacthen® stimulation was more frequently seen in patients 26/344 (7.1%) than controls 0/113 (0%) ($P=0.01$), whereas an insufficient response to ITT was seen equally frequent in patients 9/204 (4.6%) and controls 3/116 (2.6%) ($P=0.7$). An insufficient response to PD-GHRH or GHRH-arginine was seen more often in patients 47/360 (11.6%) than controls 2/93 (2.1%) ($P<0.01$), whereas an insufficient peak GH to ITT was equally frequent in patients 9/200 (4.5%) and controls 2/88 (2.3%) ($P=0.4$). A total testosterone below the lower cut-off was seen in 32/300 (10.7%) male patients vs 0/62 (0%) controls ($P=0.01$). In women, hypogonadism could not be excluded in 4/152 (2.6%) female patients vs 1/32 (3.1%) controls ($P=0.9$). Free T4 combined with an inappropriately low TSH was seen in 5/461 (1.1%) patients vs 3/96 (3.1%) controls ($P=0.1$).
Conclusion
This nationwide study of TBI patients assessed 2.5 years after the injury illustrates that the chosen methodology is very important when defining the prevalence of pituitary insufficiency. Hormonal responses in TBI patients may or may not differ from what should be expected from healthy controls.

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P850

Endoscopic transsphenoidal approach as a promising surgical option in the treatment of craniopharyngioma

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Introduction
The management of craniopharyngioma (CR) remains a challenge. The introduction of endoscopic technique in the surgical approach to the sellar/suprasellar region would have profound implications for treatment. The aim of the study was to analyze results, advantages and limits of endoscopic endonasal approach (EEA).
Design/methods
From 1998 to 2012, 95 patients underwent surgery for CR. Trancranial approach was chosen for 33 patients (35%) on the basis of shape, size, and location. The remaining 62 (52% sellar and suprasellar, 45% purely suprasellar, 3% purely intrasellar; male to female ratio: 0.73; median age: 46 years, range, 3–83 years; 26% aged 18 years or younger) were operated by EEA, for a total of 71 surgical procedures, including nine recurrences during the follow-up period (mean: 59 months, range, 3–98).
Results
At presentation, visual impairment was detected in 77%, hypopituitarism in 54%, isolated diabetes insipidus (DI) in 3%, panhypopituitarism coupled with DI in 24%. Endocrine function became further worse postoperatively, as expected (novel cases of DI and hypopituitarism occurred in 12.6 and 15.4%, respectively); conversely, after EEA visual function returned to normal in 35%, improved in 47%, and remained unchanged in 18%. Gross tumor removal was obtained in 80%. Morbidity consisted in post-operative CSF leak (18%) and chronic subdural haematoma (1.5%); one acute post-operative hydrocephalus (1.5%) was fatal. Weight gain occurred in 6%. Recurrence rate was 14.5%. Quality of life was preserved in 85% of cases, a moderate worsening (social reintegration at a lower level) occurred in 10% and a heavy worsening (semi- or totally dependent) in 5%.
Conclusions
EEA was a reliable approach in the majority of patients (65%) referred to us. The technique, safe and well tolerated, provides a direct approach along the way of the tumour growth and allows the surgeon to remove the lesion, avoiding brain retraction and vascular-nervous structures manipulation.

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P851

A precocious GH peak at GHRH plus arginine test in GH sufficient short children is predictive of a lower growth velocity

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Introduction
In children, GH secretion is considered sufficient when at least one value is >20 ng/ml at the GHRH + arginine (ARG) test. Because GH typically peaks at 45 min, we evaluated whether peak occurrence at one specific time is predictive of clinical outcomes in short stature children who are GH sufficient.
Subjects and methods
Children who performed a GHRH plus ARG test for short stature were retrospectively recruited. Inclusion criteria were: i) a GH peak >20 ng/ml; ii) Tanner stages within 1–3 stages; iii) 1 year growth velocity (GV) since the test execution; iv) born adequate for gestational age; v) the absence of signs suggestive of syndromes. Primary outcomes were height standard deviation score (SDS), GV, GVSDS and IGF-I SDS.
Results
A 228 subjects were recruited, by which 14 were excluded because they did not satisfy inclusion criteria. Of 214 subjects, 121 (56.5%) had a peak at 45 min, 55 (25.7%) at 30 min, and 38 (17.8%) at 60 min. Subjects presented a peak at 30 min had lower height SDS ($P<0.05$), GV ($P<0.001$), GV SDS ($P<0.001$), and GH peak ($P<0.05$) than those had a peak at 45 min. Subjects presented a peak at 30 min had lower GV ($P<0.001$), and GVSDS ($P<0.001$), but higher GH peak ($P<0.05$) than those had a peak at 60 min. No differences were shown between children with a peak at 45 or 60 min. No differences in Tanner stages, sex, IGF-I SDS were recorded among three groups.
Conclusion
A peak at 30 min at the GHRH + ARG test in children who are short and without GH deficiency may be predictive of lower GV in the year of the test. Because ARG infusion stops at 30 min, a somatostatinergic higher tone could have a role in the clinical picture.

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P852

A novel mutation in gonadotropin releasing hormone receptor causing delay in puberty in a sporadic case of isolated hypogonadotropic hypogonadism

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The signaling of G protein-coupled receptor 54 (GPR54) is a key regulator of the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamic GnRH neurons, whereas GnRH is a crucial neurohormone regulating the secretion of FSH and LH at the time of puberty. The deficiency in the release or action of GnRH leads to hypogonadotropic hypogonadism (HH) characterized by low FSH, LH and testosterone (T) and results in absent or impaired sexual development at puberty. In boys, the absence of the signs of sexual maturation at the age of 15 years is referred to as delayed puberty. Amongst others, mutations in GPR54 and GnRH receptor (GnRHR) are possible causes of HH. The present study was designed to determine the role of mutations in GPR54 and GnRHR genes in causing HH in Pakistani boys. Thirty-one patients with delayed puberty

and 31 normal age matched controls were included in the study. Genomic DNA was extracted and amplified by PCR using specific primers for GPR54 and GnRHR splice site exons. Mutations were analyzed by single-stranded conformation polymorphism and/or sequencing. No mutation was identified in GPR54 gene, while two mutations in GnRHR gene were observed in one sporadic case of isolated HH. One was T to G synonymous mutation at nucleotide position 123, which did not cause substitution of valine with any other amino acid. The other mutation at position 101 of the neucleotide was a missense mutation, which substituted serine with phenylalanine at 34th position of the extracellular domain of the GnRHR. This mutation, Ser34Phe, observed in 16 years old boy, caused low concentrations of FSH, LH and T and possibly delayed his puberty. In conclusion, the present study demonstrates that mutations in GnRHR may play a role in delaying male puberty in our local population.

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P853

Evaluation of late-night salivary cortisol during a Phase III study with pasireotide in patients with Cushing's disease

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Introduction
Measurement of salivary cortisol is a simple, convenient, accurate and reproducible technique with potential value during the diagnosis/management of hypercortisolism. Current analysis evaluates changes in late-night salivary cortisol (LNSC) during pasireotide treatment in patients with Cushing's disease (CD).
Methods
A 12 m, Phase III study enrolled 162 adults with confirmed *de novo* or persistent/recurrent CD who were randomized to pasireotide 600 μg/900 μg s.c. bid. LNSC assessment (assay: cortisol ELISA RE52611, IBL-Hamburg GmbH, Germany) was an exploratory objective based on a single, optional measurement at midnight $\pm$1 h on the same day as one of the 24-h UFC measurements. UFC control: UFC $\leq$ ULN; partial UFC control: UFC $>$ ULN and $\geq$50% decrease from baseline.
Results
Baseline LNSC was measured in 93 patients; median levels were 17.3 and 10.3 nmol/l in the 600 μg (n=48) and 900 μg (n=45) dose groups (normal range 0.83–8.3). After 6 m, median levels decreased by 4.9 nmol/l (26.5% (95% CI −44.8, 39.3)) and 2.4 nmol/l (41.8% (95% CI −43.7, 34.0)) in the 600 μg/900 μg groups, respectively. LNSC was normalized at 6 m in 25/67 (37.3%) patients with baseline LNSC $>$ ULN, comprising 16/40 (40.0%) and 9/27 (33.3%) patients in the 600 μg/900 μg groups, respectively. 10/25 patients with normalized LNSC at 6 m also had normalized UFC; seven had partial UFC control. Overall median LNSC decreases at 12 m were 7.2 nmol/l (42.2% (95% CI −40.8, 18.3)) and 1.6 nmol/l (26.1% (95% CI −41.1, 13.5)) in the 600 μg/900 μg groups, respectively. In both groups LNSC decreased in UFC controlled/partially controlled patients and increased in uncontrolled patients; patient numbers within each UFC control subgroup were low. An exploratory analysis demonstrated weak linear correlation (r=0.2), but moderate correlation (r=0.5) on the log scale between LNSC and UFC when all time points were pooled; the linear correlation was strong (r=0.9) at baseline.
Conclusions
Pasireotide decreased LNSC levels during 12 m of treatment. Salivary cortisol may be a simpler and more convenient biomarker than 24-h UFC to assess hypercortisolism patients with CD; further studies are required.

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P854

Evaluation of long-term pituitary functions after hypoxia due to ventricular arrhythmias: a preliminary report

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Introduction:
Traumatic brain injury (TBI), subarachnoid hemorrhage (SAH), stroke and cerebrovascular disease (CVD) are identified as risk factors for hypopituitarism. However, the mechanisms and the risk factors for the hypothalamo-pituitary dysfunction due to TBI, SAH, stroke and CVD are still unclear (1,2,3). Hypoxia, hypoxemia, neuroinflammation, autoimmunity could have impact in the pathogenesis (4). In patients with serious ventricular arrhythmias who need resuscitation, brain tissue may be exposed to short-term severe ischemia and hypoxia. However there are no data in the literature regarding pituitary dysfunction after ventricular arrhythmias.
Materials and methods
Thirty patients with ventricular arrhythmias (25 male, five female) were included in the study. Basal hormone levels were measured. To assess GH – insulin like growth factor (IGF-1) axis glucagon stimulation test was performed and 1 μg ACTH stimulation test was used for assessing hypothalamic–pituitary–adrenal axis.
Results
Four patients had secondary hypogonadism (13.3%). When the two stimulation tests and basal hormone levels were evaluated together no patient had ACTH deficiency, but glucagon stimulation test revealed GH deficiency in 8 of 30 (26.6%) patients. There were no statistical difference according to age, BMI, IGF-1 levels and other hormone levels between GH deficiency and GH sufficient group. However, IGF-1 levels were significantly lower in GH deficiency group.
Discussion
In conclusion, present data clearly suggest that in patients with serious ventricular arrhythmias who need cardiac resuscitation, pituitary dysfunction is not uncommon. Future studies need to be done to understand whether routine screening of pituitary functions in this patient group is clinically relevant or not.

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P855

Long-term efficacy of long-acting somatostatin analogues in combination with pegvisomant in 112 acromegaly patients, a retrospective single centre study with follow up for up to 8 years

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Introduction
Pegvisomant (PEGV) has an efficacy of $>$90% to control insulin-like growth factor (IGF-1), however in everyday practice a limited efficacy of 63% was reported.
Aim
To assess efficacy in the largest single center cohort of acromegalics using PEGV.
Methods
A 112 subjects (65 male) were not controlled with high-dose somatostatin analogues (LA-SRIF) for at least 6 months. To control acromegaly, 109 subjects added PEGV and three used PEGV alone. IGF-1 and GH levels (start, lowest and last values) were retrospectively re-assessed in a single run. IGF-1 was measured by the Immulite2000 and GH by the IDS-iSYS immuno-assay, to assess GH without interference of PEGV. Results are expressed as median (interquartile range). At baseline 80% of the subjects (age 47.3 years (38.4–59.0)) had a macro-adenoma.
Results
Duration of PEGV treatment was 4.0 years (1.9–6.2). Normalization of IGF-1 was observed in 96%, with an IGF-1 of 17.9 nmol/l (13.0–23.8) and a ratio IGF-1 of 0.6 (0.4–0.7). At the last visit, weekly PEGV dose of 80 mg (60–120 mg) was used during combination treatment with LA-SRIFs. In patients with mono-therapy a weekly PEGV dose of 210 mg (125–280 mg) was used. No significant differences in PEGV dose, needed to control IGF-1 were observed between sexes, diabetic/non-diabetic patients, surgery/non-surgery and Gilberts

polymorphism/non-Gilberts polymorphism.
Baseline GH levels (on LA-SRIFs) were not significantly different compared with GH during PEGV and LA-SRIFs treatment ($P=0.567$). Two subgroups were defined according to surgical status (surgery vs non-surgery). After surgery GH seem to decrease during treatment (8.7 and 4.5 nmol/l), while primary medical treatment GH seem to increase (4.0 and 4.5 nmol/l), although not significantly different ($P=0.091$ (post-radiotherapy-group was excluded)).
Conclusion
Combination of LA-SRIF and PEGV in acromegaly is effective up to 8 years, with an efficacy of 96%. GH levels do not change during treatment.
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P856

Long-term safety of long-acting somatostatin analogues in combination with pegvisomant in 133 acromegalic patients, a retrospective single centre study with follow up for up to 8 years

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Introduction
Pegvisomant (PEGV) has an efficacy of >90% to control insulin-like growth factor-1. Main safety issues are elevated transaminases that seem to be related to Gilbert's polymorphisms (GiPism) or gender and tumor-size increase (1).
Aim
To assess safety in the largest single center cohort of acromegalics using PEGV.
Methods
Results are expressed as median (interquartile-range).
One-hundred and thirty-three acromegaly patients (73 males) used PEGV and long-acting somatostatin analogues LA-SRIF, to control active disease ($n=112$) or to enhance quality of life ($n=21$), over a period of 4.0 years (1.9–6.2).
Results
At baseline, 79% of the subjects (age, 49.1 years (39.3–59.2)) had a macro-adenoma. Transient dose independent elevated transaminases (TDIET) of more than three times the upper limit of normal (>3x ULN) were observed in 20 patients (15%), they resolved without PEGV dose adaptation. One patient discontinued PEGV, as previously reported (2). Biliary tract disease could explain at least two of these cases, so 14% could be linked to PEGV use. TDIET >3x ULN occurred after 5.2 months (3.0–15.9) and normalised in 3.9 months (2.8–5.1). Re-exposure to PEGV after discontinuation resulted in a second period of TDIET in two patients.
GiPism was found in 68 (54%) of 122 tested patients, 11% homozygous and 45% heterozygous. Of the 20 TDIET cases, three (15%) were homozygous and seven (35%) were heterozygous. No association between GiPism and developing TDIET was found in patients with heterozygous ($P=0.59$) or homozygous polymorphism ($P=0.36$) compared with non-GiPism or gender ($P=0.08$).
Tumour-size decrease was observed in 12% but size could not be evaluated in 14 patients due to an empty sella. One patient needed surgery due to tumour-size increase.
Conclusion
Combination of LA-SRIF and PEGV in acromegaly is safe up to 8 years. TDIET are observed in 15% of acromegalics. TDIET are not related to GiPism or gender. In 12% tumour size decrease occurred.
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P857

Similar response to therapy of pituitary adenomas with and without SOX2-expressing cells

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Introduction
SOX2 is an early developmental transcription factor and a marker for pituitary progenitor cells.
The study aimed to investigate if the pituitary adenomas with positive SOX2 immunoreactivity shows a different response to therapy as compared with controls.
Patients and methods
We investigated 15 pituitary macroadenomas, eight with SOX2 immunoreactivity (SOX2+) and seven without (SOX2−) (controls); five were GH producing tumours (three SOX2+, two SOX2−), five prolactinomas (four SOX2+, one SOX2−) and five non-functioning pituitary tumours (one SOX2+, four SOX2−) confirmed by immunohistochemistry, ABC method. Anterior pituitary hormones were measured in simultaneously sampled serum and cerebrospinal fluid (CSF) by fluoroimmunoassay with europium. Three patients with SOX+ and three SOX− adenomas showed high CSF:serum ratio for one or two hormones (five of them for FSH and/or LH and one for prolactin). The tumour volume was appreciated by CT or MRI.
Results
All pituitary adenomas were partially removed by surgery. Additional treatment were i) high-voltage radiotherapy or gamma knife (two SOX+ and three SOX− tumours); ii) radiotherapy associated with medical treatment with somatostatin analogs and/or dopaminergic agonist cabergoline (four SOX+ and one SOX− tumours); iii) only medical treatment (one SOX+ and two SOX− tumours). The average follow-up was 53.3 months.
From first group A with radiotherapy, both SOX2+ pituitary adenomas were cured and there was upper 50% decrease in tumour volume in two SOX2− pituitary adenomas. Patients with triple therapy (B) need high doses of dopaminergic agonists and somatostatin analogs. Both, tumour volume and hormone secretion decreased when drug therapy started after radiotherapy (the tumour volume decreased more than 50% in two SOX+ and one SOX− tumour and lower than 50% in two SOX+ adenomas).
Conclusion
Pituitary macroadenomas with SOX2-positive cells are not refractory to treatment and showed similar responses to radiotherapy or/and medication (somatostatin analogues or cabergoline) as the controls.
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P858

The clinical characteristics of pain in patients with pituitary adenomas

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Introduction
Clinical presentation of pituitary adenomas frequently involves pain, particularly headache, probably due to both structural and functional properties of the tumour.
Design
In a retrospective analysis, we investigated clinical characteristics of pain in 277 patients with pituitary disease ($n=81$ acromegaly; $n=50$ Cushing's disease; $n=86$ prolactinoma; $n=60$ non-functioning pituitary adenoma). Specific pain patterns were measured using three standardized pain questionnaires, MIDAS (Migraine Disability Assessment), the painDETECT and the DGSS (German Society for the Study of Pain) questionnaire.
Results
For the whole group, 156 tumours were macroadenomas (56%) and 121 were microadenomas (44%). Cavernous sinus invasion was observed in 55 tumours (20%). The commonest tumour associated with pain was adrenocorticotropic adenoma ($n=17$; 34%), followed by growth hormone secreting ($n=23$; 28%), prolactinoma ($n=20$; 23%) and non-functioning pituitary adenoma ($n=8$; 13%). Primary pain site was located in the lower back/bottom region (68.2%) and in the mouth/face/head region (61.9%). Regarding pain quality and severity, the majority of the pituitary patients (89%) described their pain as 'deep' in contrast to 'surface' pain and reported on a scale of 0 (=no pain) to 10 (=most severe pain) a median pain intensity of 4 (IQR 3.0–6.0) within the last 4 weeks. Pituitary patients presented primarily with an episodic (46%) followed by a permanent/chronic (30%) pain component. Most common pain-associated features comprised noise sensitivity (67.9%), visual disturbances (64%), photophobia (56%) and nausea (53.8%). Both physical (81.4%) and emotional stress (46.5%) were reported to trigger pain. Tumour-associated headache leads to severe levels of disability in daily life in a minority of patients (13.5%).
Conclusion
Pain appears to be a significant problem in pituitary disease and is associated with a range of pain phenotypes. A combination of factors including tumour activity, cavernous sinus invasion, as well as previous predisposition to pain might play a role in pain phenotypes.

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P859

Genetic and clinical characteristics of Serbian FIPA families

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Introduction
Recently, major advances have been made in genetics of familial acromegaly and isolated pituitary adenoma (FIPA). Mutations in the aryl hydrocarbon interacting protein (AIP) gene have been found in 25–50% of patients with FIPA and familial acromegaly.
Aim of the study
Our goal was to identify and collect data on patients who met the criteria for FIPA.
Patients and methods
The patients were identified between 2008–2012 in the Clinical Center of Serbia, Belgrade. Genetic analysis of AIP gene was performed as part of the International FIPA Consortium project. All patients signed informed consent for genetic testing before entering the study.
Results
In 24 families, 51 subjects (31F/20M) with FIPA were identified. Four GH families (two with gigantism), four PRL, two NFPA, five NFPA-PRL, three GH-PRL, four GH-NFPA, one GH-PRL-NFPA and one PRL-ACTH were registered. Average age at diagnosis for patients with mutations in the AIP gene was 26 ± 23 years compared to 38 ± 12 years for AIP mutation-negative group. Median age at diagnosis was 14 for gigantism, 32 for prolactinoma, 35 for acromegaly and 50 for NFPA. In male patients, 75% were macroadenomas compared to 45% in female patients. So far, 15 families have been screened for AIP mutations. In 13% (two patients with gigantism and one with NFPA), AIP mutations/deletions were registered.
Conclusion
So far, our results indicate that prevalence of AIP gene mutations in Serbian FIPA patients is around 13%. Further genetic testing is needed for AIP gene mutations. Other genetic causes, which may be involved also need to be investigated.

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P860

Doubled mortality ratio in female patients with non-functioning pituitary adenomas

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Introduction
Patients with non-functioning pituitary adenomas (NFPA) and hipopituitarism had a reduced life expectancy.
Aims
To assess mortality ratio and to identify prognostic factors associated with mortality in patients with NFPA in the last decade.
Methods
A 196 patients (83 F/113 M, mean age 52.7 ± 0.9 years) with NFPA admitted in a single Neuroendocrinology Department between January 2001 and December 2010 were retrospectively studied. PAMCOMP computation program was used to calculate standardized mortality ratio (SMR). Cox regression analysis revealed independent factors associated with mortality.
Results
During follow-up (median 7.5 years–1298.04 person years), 26 patients died, corresponding to a death rate of 20 deaths/1000 person years. Statistically significant more patients with hypopituitarism for at least one axis deceased (22/156, 14.10%), as compared with patients without pituitary failure (1/22 patient deceased – 2.07%), $P=0.03$. All causes mortality was not statistically different from that of general population: Standardized mortality ratio (SMR) was 1.2 (95% confidence interval (CI) 0.83–1.86). Females had a doubled mortality ratio: SMR 2.03 (95% CI 1.01–3.64), but males had a mortality ratio similar to general population: SMR 0.87 (95% CI 0.48–1.44). When assessed by Cox-regression analysis, prednisone dose for corticotrophin insufficiency (HR 1.5 (95%CI 1.18–1.98)) and last systolic blood pressure (HR 1.04 (95%CI 1.005–1.081)) were independent predictors of mortality in females. Mortality rose progressively with prednisone dose (log rank: $P=0.007$) in females, but not in males (log rank: $P=0.197$).
Conclusions
Females with non-functioning pituitary adenomas and hypopituitarism had a doubled mortality ratio as compared with general population, influenced by prednisone dose used for substitution and systolic blood pressure.

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P861

Incidentally discovered pituitary adenomas: single-center experience on 205 patients

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Introduction
Pituitary incidentalomas are lesions discovered on an imaging study performed for an unrelated reason. Their frequency varies among 0.2–38% and it is continuously increasing due to the development of neuroimaging techniques. The aim of the study is to investigate clinical and biochemical characteristics of 205 consecutive patients (70% female, mean age 53.6 ± 18.2 years) with incidental pituitary adenoma (IPA) followed at our center from 1990 to present.
Methods
In all patients, hormonal evaluation (basal anteropituitary function, ACTH 250 mg stimulatory test, other dynamic tests when indicated) and pituitary imaging were performed at baseline, 6 months later and then annually if there was

no other specific indication. Seventy patients were also screened for subclinical hypercortisolism (cortisol after 1 mg overnight dexamethasone suppression test (1 mg DST), late-night salivary cortisol, 24-hours urinary free cortisol).
Results
At diagnosis, 39% of patients had macroadenomas. One or more pituitary deficiencies were observed in 14.3% of cases (macro 28.6 vs micro 5.6%, $P<0.05$). Hyperprolactinemia (<100 ng/ml) was observed in 13.9% of patients (macro 15.5 vs micro 12.8%, P NS). Subclinical hypercortisolism was found in 3/70 (4.2%) patients studied, all with macroadenomas. One hundred and ten patients had a follow-up longer than 12 months with a mean follow-up of 4.9 years. Radiological evaluation revealed a significant increase in tumor mass in 19/110 patients (17.3%, 13 macro vs 6 micro, P NS) and a reduction in 5.4% (all microadenomas). The volumetric increase occurred in 88% of patients during the first 2 years after diagnosis. Additional pituitary deficiencies were observed in 3% of patients during follow-up. Overall, 18% of patients were treated with transsphenoidal adenomectomy owing to initial mass size or for their rapid increase.
Conclusions
Our data confirm that patients with IPA need for a close radiological and hormonal follow-up. In addition, we suggest exclusion of subclinical hypercortisolism in such patients.

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P862

Clinical diagnostic implications of sequential pattern of dynamic MRI for pituitary microadenoma

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Objective
We examined whether sequential enhancement patterns of pituitary microadenomas on dynamic MRI correlates with different subtypes of pituitary tumors.
Materials and methods
Patients with pituitary microadenomas imaged via dynamic MRI between years 2000 and 2012 were included. The intensity of the adenoma and of normal gland at each time point in the dynamic sequence was measured by drawing a region of interest (ROI) on both the normal pituitary gland and the tumor. Measurements included: enhancement ratio (ER), defined as the ratio of the tissue at time (t) vs the baseline ($t=0$); pituitary-adenoma ratio (PAR), which is the ratio of the normal anterior pituitary lobe to that of pituitary adenoma; and, the speed to reach the peak intensity. From these data tumors were classified as early, simultaneous or late (peak before, at the same time or after normal pituitary tissue, respectively). Correlation between the enhancement pattern and the tumor subtype was analyzed using Pearson product-moment correlation coefficients and logistic regression analysis.
Results
A total of 118 patients had functional adenomas, while 47 patients had nonfunctional adenomas. We found that the enhancement levels in all pituitary adenomas subtypes were lower than those in normal pituitary tissue ($P<0.0001$). There was a significant correlation between the enhancement patterns of functioning vs nonfunctioning tumors ($P=0.0397$) and between the four tumor subtypes ($P=0.0039$). Functional adenomas usually exhibited simultaneous or late enhancement pattern while nonfunctional adenomas showed early enhancement pattern. The time to reach peak enhancement in normal pituitary tissue was shorter nonfunctioning adenomas ($P=0.0082$) and GH tumor group ($P=0.0042$).
Conclusion
Our study is the first to show significant correlation between enhancement pattern and pituitary adenoma subtype. This finding can provide supplemental information in differential diagnosis of pituitary microadenomas as well as guide future studies of appropriate timing of image acquisition according to tumor subtypes.

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P863

How to get surgical remission rates in ACTH- and GH-microadenomas to 100%

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Introduction
Pharmacological treatments are available for all functioning pituitary adenomas, but found to be only ideal in prolactinomas. Therefore, at least in ACTH- and GH-microadenomas we have to strive to achieve 100% remission with transnasal surgery. Our question is, which combination of an increasing arsenal of pre- and peri-operative methods will bring the pituitary surgeons to this result without increasing complications.
Materials and methods
Data from published series of the author will be compared with new data from recent series. All patients had been operated, when MRI and direct transnasal micro-surgery were established. Special diagnostic methods: pretreatment with somatostatins and GH-receptor blocker; intra- and early postoperative hormone measurements; intra-operative micro-histology; micro-doppler; neuro-navigation; as well as the importance of a micro-suction irrigation system for visualization and minimization of trauma, will be described and illustrated.
Results
ACTH-micro-adenomas: since introducing more refined diagnostic tests and cavernous sinus sampling, nearly all micro-adenomas were initially detected. Nevertheless, in a few cases early re-surgeries became necessary to achieve remissions. This was especially evident and important in children with Cushing ($n=100$).
GH-microadenomas: in 2001 we published 100% remission in patients pretreated with somatostatin analogs vs 93% without pretreatment. This was statistically not significant, but of importance for the patients. Only rarely were intra-operative GH measurements influential in the group of microadenomas. In the last series from 2001–2005 without intra-operative GH-measurement and a long follow-up (mean >5 years), a clear biochemical remission was surgically achieved in all 25 microadenomas. The majority was pretreated with somatostatins. Complications were rare, minor and transient.
Conclusions
Using advanced surgical micro-techniques, enhanced by immediate feedback of the surgical success with hormone measurements, our long-term remission rates are definitely superior to most published data with medical and/or radio-surgical treatment. There is a great benefit for patients and minimizes costs.

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P864

Moderate hyponatremia is associated with an increased risk of overall mortality: a comprehensive meta-analysis

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Introduction
Hyponatremia is the most common electrolyte disorder in clinical practice, and evidence to date indicates that severe hyponatremia is associated with increased morbidity and mortality. The aim of our study was to perform a comprehensive meta-analysis that included all the published studies that compared mortality rates in subjects with or without hyponatremia of any degree.
Methods
An extensive Medline, Embase, and Cochrane search was performed to retrieve all studies published up to October 1, 2012 using the words 'hyponatremia' and 'mortality'.
Results
Eighty-one studies satisfied inclusion criteria encompassing a total of 850 222 patients, of whom 147 948 (17.4%) were hyponatremic. Across all 81 studies, hyponatremia was significantly associated with an increased risk of overall mortality (RR=2.60 (2.31–2.93)). Hyponatremia was also found to increase the risk of mortality in patients with multiple diseases, including myocardial infarction (RR=2.83 (2.23–3.58)), heart failure (RR=2.47 (2.09–2.92)), cirrhosis (RR=3.34 (1.91–5.83)), pulmonary infections (RR=2.49 (1.44–4.30)), mixed diseases (RR=2.50 (1.97–3.18)), and in hospitalized patients in whom the diagnosis was not specified (RR=2.48 (2.09–2.45)). A mean difference of serum (Na+) of 4.8 mmol/l was found in subjects who eventually died compared to survivors (130.1±5.6 vs 134.9±5.1 mmol/l, $P<0.001$). Furthermore, a meta-regression analysis showed that the hyponatremia-related risk of overall mortality was inversely correlated with serum (Na+) (S=−0.096 (−0.114, −0.077); I=13–710 (12.258–16.161); both $P<0.0001$). This association was confirmed in a multiple regression model after adjusting for age, sex, and associated morbidities such as diabetes mellitus.

Conclusions
This meta-analysis shows for the first time that even moderate serum (Na+) decreases are associated with an increased risk of mortality in commonly observed clinical conditions across large numbers of patients.

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P865

Effects of somatostatin analogues on muscle sympathetic nerve activity in acromegaly

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Introduction
While searching for mechanisms contributing to the increased mortality of acromegaly, we have previously described an unexpected sympathoinhibition in newly diagnosed patients (Seravalle *et al.*, Clin Endocrinol 77:262, 2012) and interpreted this finding as an adaptive phenomenon. It has also been shown that centrally administered somatostatin (SS) inhibits peripheral sympathetic outflow in rodents (Rettig *et al.*, Am J Physiol 257:R588, 1989). Based on the above, we elected to study muscle sympathetic nerve activity (MSNA) in acromegalic patients before and during treatment with SS analogues (SSA).
Study
MSNA was directly measured by microneurography in the following groups of subjects: i) 24 newly diagnosed acromegalics (13 men and 11 women, mean age 45.5±13.0 years); ii) 22 patients on SSA, 11 of whom (seven men and four women, mean age 52.4±13.9 years) attaining biochemical control according to the currently accepted criteria and 11 (five men and six women, mean age 56.4 ±17.5 years) not attaining biochemical control; iii) 17 normal-weight healthy subjects serving as controls (11 men and six women, mean age 49.1±15.6 years).
Results
As expected, mean MSNA was significantly lower in untreated acromegalic patients than in control subjects (18.3±8.39 vs 37.8±6.60 bursts/min, $P<0.01$). Patients on SSA, either with controlled or uncontrolled disease, displayed mean MSNA values (27.4±8.24 and 31.6±3.27 bursts/min respectively) significantly lower than those shown by controls ($P<0.01$) but significantly higher than those found in untreated acromegalics ($P<0.05$). Mean MSNA values were not significantly different between controlled and uncontrolled SSA-treated patients.
Comment
The present study has confirmed the profound sympathoinhibition characterizing untreated acromegaly and has shown the reversibility of this alteration with the improvement of the disease. These preliminary data do not allow to unveil a possible role of SSA in these changes.

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P866

Effects of short (12 months) and long (60 months) term treatment with cabergoline on metabolic syndrome and visceral adiposity index in patients with hyperprolactinemia

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Introduction
Hyperprolactinemia is reportedly associated with an impaired metabolic profile, particularly in patients with concomitant hypogonadism. The current study aimed at investigating the effects of short (12 months) and long (60 months) treatment with cabergoline (CAB) on metabolic complications, metabolic syndrome (MS) prevalence and visceral adiposity index (VAI) in hyperprolactinemic patients.
Patients and methods
Seventy-one patients (51 F, 20 M, aged 35.4±11.7 years), including 36 with microprolactinomas, 32 with macroprolactinomas and three with non-tumoral hyperprolactinemia entered the study. In all patients, PRL and metabolic parameters (BMI, waist circumference, lipid and glucose profile, insulin, VAI) were assessed at diagnosis and after 12 and 60 months of continuous CAB treatment. MS was evaluated in line with NCEP-ATP III criteria.
Results
Compared to baseline, CAB induced a significant decrease in PRL levels after 12 months ($P=0.000$) and a further decrease after 60 months ($P=0.000$) with complete normalization in 93% of patients. At baseline, MS prevalence was significantly higher in patients with PRL above than in those with PRL lower than the median (187 µg/l) ($P=0.02$). MS prevalence significantly decreased after 12 (12.6%, $P=0.009$) and 60 (7%, $P=0.000$) months of treatment compared to baseline (32.4%). Total cholesterol and triglycerides were significantly reduced after 12-month CAB compared to baseline ($P=0.03$), and further decreased ($P=0.000$) after 60-month follow-up. HDL-cholesterol resulted significantly increased after 60-month CAB compared to baseline ($P=0.000$) and 12 months ($P=0.000$). Glucose and insulin levels significantly decreased after 12 months of CAB ($P=0.001$) and were further improved after long-term CAB ($P=0.03$ and $P=0.000$ respectively) compared to short-term therapy. Compared to baseline, a slight but not significant decrease in VAI was found at 12-month evaluation, whereas VAI was significantly decreased after 60 months of treatment ($P=0.000$).
Conclusions
Short-term CAB treatment significantly improves metabolic profile, so that to reduce MS prevalence, whereas longer treatment is required to achieve a significant improvement of VAI.

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P867

Real world data in acromegaly – a retrospective chart audit

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Introduction
The ideal strategy and the role of the different therapeutic options in acromegaly remain unclear. This research focuses on observing treatment options, and the respective level of disease control in acromegaly.
Methods
A retrospective chart audit was conducted in US, France, Italy, and Brazil (September 2012). 97 endocrinologists completed structured case report forms for the 4 most recently-seen patients (two with somatostatin analogues (SSAs), two with pegvisomant (PegV) or PegV+SSAs), $n=380$; those on the last choice of medical therapy ≧6 months were included in the analysis ($n=335$). Patient demographics, comorbidities, treatment history, insulin-like growth factor type I (IGF-I) expressed as ULN-fold of age-related reference range, and symptoms were collected. Here we report the preliminary data related to disease management and IGF-I control.
Results
Of the 335 medically treated patients (mean age 51 yrs; 52% males), 193(58%) were treated with SSAs, 116(35%) with PegV, and 26(8%) with PegV+SSAs. 208 of 335 patients (62%) were treated with surgery prior to medical therapy (74% US, 63% Brazil, 54% France, 51% Italy), 127 (38%) received medical therapy with ($n=6$) or without ($n=121$) radiation. 63 of 208 (30%) patients treated with surgery prior to medical therapy and 66 of 127 (52%) treated with medical therapy had IGF-1>ULN. 20% of all study patients had IGF-1>1.5xULN (19% US, 28% Brazil, 21% France, 15% Italy) despite treatments. 59% of SSA treated patients had IGF-1≤ULN, vs. 67% of patients on PegV and 73% on PegV+SSAs. The rates of acromegaly symptoms and comorbidities were similar among patients treated with SSAs and PegV. Incidence of paresthesia and perspiration were significantly lower in patients with IGF-1≤ULN ($P<0.05$).
Conclusions
Medical therapy remains an integral component of acromegaly management. Despite the different therapeutic strategies, including different drugs, 30-52% of

patients remain with elevated IGF-I and may experience signs and symptoms of active disease.

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P868

Prevalence of escape and lipodystrophy in patients treated with Pegvisomant. A multicenter study

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Pegvisomant (peg) is an effective treatment for acromegaly.

Aim

To investigate the prevalence of escape and the incidence of lipodystrophy with peg treatment.

Methods

Multicenter retrospective study. Escape was defined as loss of control in patients previously controlled under a stable dose of peg, without any other treatment change. Lipodystrophy was defined as either hypertrophy or atrophy of subcutaneous tissue in areas of drug administration.

Results

Ninety-eight patients were included (59% women, mean age at diagnosis 42 ± 13 years, 80% macroadenomas). Ninety-two percent achieved normal IGF-1 on peg. Escape was reported in 33/90 (37%) of responders. Mean time on peg before escape was 22 ± 20 months. The mean initial dose was 11 ± 3 mg/day and mean dose at escape was 14 ± 7 mg/day. Most patients: 30/33 (91%) achieved control with dose increase (57%), medical treatment addition (13%) or both (27%); in one case, escape was temporary. Treatments associated were cabergoline in 50% somatostatin analogs in 45% and both in 5%. Mean new dose that controlled IGF-1 after escape was 19 ± 8 mg/day. Lipohypertrophy was observed in 14 patients (14.4%) whereas lipoatrophy was observed in one (1%). Lipohypertrophy developed in the abdominal wall in four, arms in two, thighs in four and in multiple sites in four cases and was persistent over time in 30%. Due to lipohypertrophy, peg was discontinued in four patients, four had dose reductions and in six no action was taken. There was no correlation between the presence of escape and lipodystropy, as well as with multiple clinical variables.

Conclusions

Pegvisomant is an effective treatment for acromegaly, although 37% of responders escaped in long-term follow-up. Most patients achieved control with either dose increase, other medical treatment addition or both. Lipohypertrophy developed in 14% of patients, was persistent in 30% of cases and required drug discontinuation in 28% (four of 14).

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P869

The effect of the ANKK1/DRD2 Taq1A polymorphism on metabolic side effects of dopaminergic treatment in PRL adenomas

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Introduction

Treatment with dopamine agonists in patients with PRL adenomas has been associated with weight loss in short-term studies. However, long-term studies on weight changes are lacking. Taq1A is a restriction fragment length polymorphism considered as a gene marker for the *D2DR* gene. The presence of at least one A1 allele is linked to reduced brain dopaminergic activity due to reduced receptor binding and lower density of the dopamine two receptor.

Objectives

We aimed at testing the hypothesis that the dopaminergic treatment in prolactinoma patients leads to sustained weight loss and that the presence of diminished weight loss response under dopamine agonists is associated with the minor A1 allele of *Taq1A*.

Materials and methods

We included $n = 44$ patients (17 males and 27 females, 26 macroadenomas and 18 microadenomas) with PRL adenomas treated with dopamine agonists (cabergoline, bromocriptine, quinagolide, and metergoline) into this study. Outcome measures were weight and body mass index (BMI) change under dopaminergic treatment after 2 years with regard to *Taq1A* status.

Results

We observed that the dopaminergic treatment leads to a significant mean weight loss of 3.1 ± 6.25 kg after 2 years. Regarding *Taq1A* polymorphisms, 21 patients were carriers of at least one A1 allele (genotype A1/A1 or A1/A2) and 23 patients had a genotype of A2/A2. However, the presence of the A1 allele was neither associated with the mean BMI at baseline nor with an altered weight loss response under dopamine agonist therapy.

Discussion

Our results implicate that the dopaminergic treatment leads to a sustained weight loss in patients with PRL adenomas after 2 years. However, there was no association to the A1 allele of *Taq1A*, observation that needs to be analysed in larger cohorts.

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P870

Incidental haemorrhage in prolactinomas: is it of any clinical significance?

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Background

Incidental pituitary haemorrhage in prolactinomas is a common radiological finding. The clinical significance, associations and outcome of this are largely unknown. Most reports describe surgically treated macroprolactinoma and non-functioning adenoma, and there are few data in a clinic prolactinoma population.

Aims

To characterise the prevalence, natural history and risk factors associated with pituitary haemorrhage in a large clinic prolactinoma population.

Method

A retrospective case-note analysis of 368 patients with prolactinoma attending Guy's and St. Thomas' Hospitals between 2000 and 2008. Presence of haemorrhage was noted on magnetic resonance imaging (MRI).

Results

Pituitary haemorrhage was found in 25 patients, giving an overall prevalence of 6.8% and was significantly more prevalent in macroprolactinoma (20.3%) than in microprolactinoma (3.1%) ($P < 0.0001$). Three patients had classical pituitary apoplexy. The majority of patients in the haemorrhage group had macroprolactinomas (16/25 (64%)) and the majority were female (22/25 (88%)). The proportion of females with macroprolactinoma was also higher in the haemorrhage group (14/16 macroprolactinomas (87.5%)) than in the non-haemorrhage group (36/63 macroprolactinomas (57.1%), $P = 0.02$).The majority of patients were treated conservatively (92%) with 87% of patients having complete resolution of their haemorrhage within 26.6 ± 5.2 (mean $\pm$ standard

error of mean) months. Anticoagulant therapy, diabetes, hypertension and type of dopamine agonist therapy were not associated with pituitary haemorrhage. After adjustment for confounders, the presence of macroprolactinoma (odds ratio 9.00 95%CI 3.79–23.88, $P<0.0001$) and being female (odds ratio 8.03 (95% CI 1.22–52.95, $P=0.03$) were independently associated with haemorrhage.
Conclusion
These data show that haemorrhage is relatively common in macroprolactinoma where one in five develop haemorrhage but is also present in microprolactinoma. The vast majority were clinically silent and resolved spontaneously with only dopamine agonist therapy. We present novel data showing that women, particularly with macroprolactinoma, were more likely to develop haemorrhage in comparison to men.

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P871

Incidence of pituitary adenomas in Western Sweden in 2001–2011

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Background
The number of studies on the incidence of pituitary adenomas (PAs) is limited. The aim of this study was to evaluate the annual incidence of PAs in Västra Götaland, Sweden, with a targeted population of 1.6 million inhabitants.
Patients and methods
Data from adult patients diagnosed with PA in 2001–2011, living in the Västra Götaland County, were collected from the Swedish Pituitary Registry (SPR). In addition, medical records on all patients diagnosed with PA at the six hospitals in the region were reviewed. Those patients who fulfilled a predefined criteria of PA, and had not been registered in the SPR previously, were also included in the analysis. In total, 592 patients were included in the study. Age standardized incidence rate (SIR), given as rate/100 000 inhabitants (95% confidence intervals), was calculated using the WHO 2000 standard population as a reference.
Results
The total SIR for PA during the study period was 3.9/100 000 (3.6–4.3). The SIR in men was 3.3/100 000 (2.9–3.7) and increased with increasing age. In women, the SIR was 4.7/100 000 (4.1–5.3) with the highest incidence in the ages 25–34 years, corresponding to the high frequency of prolactinomas. NFPA was the most common PA (54.1%) with SIR of 1.8/100 000 (1.6–2.0). Prolactinomas were detected in 32% (SIR 1.6/100 000 (1.3–1.9)), acromegaly in 9% (SIR 0.35/100 000 (0.25–0.45)), Cushing's disease in 4% (SIR 0.18/100 000 (0.11–0.25)) and TSH-producing adenomas in 0.7% (SIR 0.03/100 000 (0.00–0.05)). The frequency of macroadenomas for NFPA, prolactinomas, GH-, ACTH- and TSH-producing adenomas were 82, 37, 77, 28, and 100%, respectively. The life-time risk of PAs in men was 0.27% (0.24–0.31) and in women 0.29% (0.26–0.33).
Conclusion
Hereby, the largest study on the incidence of PAs is presented. The study, conducted during times of frequent use of medical imaging, confirms an increased incidence of PAs compared to older studies.

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P872

Ursodeoxycholic acid role in chronic cholecystitis progression prevention in acromegaly patients receiving somatostatin analogues

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Introduction
Acromegaly – a serious debilitating disease resulting in decreased quality and length of life. Due to the late nature of the diagnosis, the lack of prospects for a surgical or non-radical adenomectomy leads to a somatostatin analogues (SA) treatment as primary or secondary therapy, which is a long-term, sometimes lifelong. Therefore, the question of tolerability and prevention of life-threatening complications for these patients is very important. Since the development of gallstones is an expected side effect of long-term use of SA, the authors decided to test the effectiveness of ursodeoxycholic acid (UA) to prevent and cure gallstone patients receiving SA.
Description of methods/design
In a 3-year observation was attended by 44 patients (11 men and 33 women) with acromegaly treated with SA and concomitant chronic cholecystitis. Thirteen patients at baseline revealed gallbladder stones and 31 biliary sludge. Twenty-eight patients received the UA (group 1) and the remaining 16 for various reasons did not receive drug therapy (group 2). Groups did not differ by sex, age (54.2 $\pm$11.4 and 58.8 $\pm$ 10.6), level of IGF-1 (703.8 $\pm$ 369.9 and 676.9 $\pm$ 300.8 ng/ml) and GH (3.1 $\pm$ 4.4 and 6.1 $\pm$ 11.7 ng/ml) and prevalence of cholelithiasis and biliary sludge. Initial dose was 500 mg UA at night and adjusted every 6 months.
Results
In group 1, none of the patients developed acute cholecystitis, 31% of patients in group 2 developed acute cholecystitis. A negative correlation between UA treatment and acute cholecystitis development in the total group was shown ($r=-0.45$, $P<0.05$). And also noted a negative correlation between the success of therapy and duration of the active phase of acromegaly in patients history ($r=-0.89$, $P<0.05$).
Conclusion
i) Permanent treatment with UA effectively prevent cholecystitis progression in acromegaly patients with SA treatment. ii) Prevention of surgical treatment increase quality of life.

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P873

Relationship between telomere length and dyslipidemia in patients with Cushing's syndrome

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Introduction
Cushing's syndrome (CS) is associated with increased mortality and morbidities. Hypercortisolism is also present in chronic mood disorders (CMD) and stress, where telomere length (TL) has been found to be shorter than in matched controls. Since hypercortisolism is present in CS and CMD, we hypothesized that telomere shortening could also be present in CS. The aim of this study was to investigate TL in CS patients compared to matched controls.
Methods/design
Transversal study. Thirty-four CS (seven males, 26 pituitary and eight adrenal; six with active disease and 28 cured from hypercortisolism) and 34 matched control (for age, gender, smoking). Mean age was 48 + 13.7 in CS vs 48.09 + 13.5 years in controls (ns). Manual DNA extraction from leukocytes using the phenol/chloroform method was performed. Leukocyte TL was measured by TRF-Southern technique (kit-telo TTAGGG Telomere length Assay, Roche).
Results
CS patients had a greater waist:hip ratio than controls (0.91 $\pm$ 0.07 vs 0.84 $\pm$ 0.08, $P<0.01$), more prevalence of hypertension (47 vs 11%, $P<0.001$) and osteoporosis (29 vs 11%, $P<0.05$). No other baseline differences were found. TL did not differ in CS (7788 $\pm$ 752 base pairs –bp vs 7446 $\pm$ 1100 bp) compared to controls. CS with dyslipidemia ($n=12$, 35%; two with active hypercortisolism) had shorter telomers than those without (7248 $\pm$ 715 bp vs 8060 $\pm$ 865 bp, $P<0.05$). No correlation was found with concomitant cortisol values, length of exposure to hypercortisolism or endocrine cure.
Conclusion
In this small group of matched CS/controls, we did not find any differences in TL; however, in CS with dyslipidemia, TL was shortened compared to CS patients with normal lipid values. Further studies will be necessary to confirm this finding and define any possible relationship between hypercortisolism and TL.
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Factors Affecting Prognosis in a Series of Acromegalic Patients

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Introduction
The main goal in the treatment of acromegaly is achieving biochemical and tumor mass control. Therapeutic options include surgery, medical treatment and, in selected cases, radiotherapy. A GH receptor (GHR) variant that differs for the genomic retention or exclusion (d3GHR) of exon 3 is present in about 50% of general population and its presence is related to a greater sensitivity to GH.
Materials and methods
We describe a series of 118 patients diagnosed with GH-secreting pituitary adenoma (24 microadenoma, 94 macroadenoma), all submitted to surgery as first-line treatment. All patients with persistent disease after surgery have been treated with somatostatin analogues (SSA) and, among these, non-responder patients have been treated with pegvisomant. We analyzed GH and IGF-1 levels, tumor size and invasiveness, Ki-67 labeling and GHR polymorphism and correlated these findings with prognosis and response to medical treatment.
Results
Twenty-eight/118 patients (23.7%) were considered biochemically cured after surgery: these patients had more frequently microadenomas (35 vs 65%) with lower Ki-67 (1.2 vs 1.7). Among the 90 patients treated with SSA, 64 (71.1%) were biochemically controlled. Patients with disease resistant to SSA presented more frequently cavernous sinus involvement (65 vs 29%) and higher Ki-67 (2.4 vs 1.5%) compared to SSA responsive patients. Twenty-six patients have been treated with pegvisomant: 18 patients (69.3%) presented biochemical control, five patients achieved normal levels of IGF-1 with further 'escape' and three patients were considered not controlled. These three patients presented with higher IGF-1 levels (828 vs 439 ng/ml) and more frequent cavernous sinus involvement (100 vs 77%) compared to controlled ones and they all lacked the d3GHR polymorphism.
Conclusion
Our data show that tumor size, local invasiveness and Ki-67 labeling are all prognostic factors in pituitary GH-secreting adenomas. Moreover, lower IGF-1 levels and the presence of the d3GHR polymorphism seem to be correlated with good response to pegvisomant.

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P875

Thirty-day mortality in acute non-surgical patients admitted with hyponatremia

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Introduction
Hyponatremia (serum sodium $\leq$135 mmol/l), the most common electrolyte disorder encountered, has been associated with increased mortality in patients with particularly cancer, heart failure, chronic kidney and liver disease. However, evidence of the clinical implications in broader populations is scarce, and uncertain due to confounding from preexisting disease. We aimed to examine the association between admission-hyponatremia and 30-day mortality in acute non-surgical patients.
Method
We conducted a population-based cohort study in North and Central Denmark Regions, comprising approximately 1.8 million inhabitants. We identified all patients acutely admitted to non-surgical departments from January 1, 2000 to December 31, 2009, for whom serum sodium was measured on the day of admission using individual level linkage of the Danish National Patients Registry, the Danish Civil Registration System and the Clinical Laboratory Information System. Admission-hyponatremia was categorized as mild (130–135 mmol/l), moderate (125–129.9 mmol/l) and severe ($<$125 mmol/l). Thirty-day mortality for normonatremia and levels of admission-hyponatremia were estimated using the Kaplan–Meier method. Mortality rate ratios (MRRs) were estimated using a Cox regression model, adjusting for sex, age and comorbidity level.
Results
We identified 302,311 acute non-surgical patients, with a sodium measurement on the first day of admission. Admission-hyponatremia was present in 70140 patients (prevalence = 23%). Hyponatremic patients were older and had higher comorbidity levels than normonatremic patients. Cumulative 30-day mortality for patients with admission-hyponatremia was 8.8% compared with 3.9% in normonatremic patients. Mortality was increased throughout the period. Adjusted MRR was 1.78 (95% CI: 1.72–1.84) for any hyponatremia compared with normonatremia. Adjusted MRR for mild, moderate and severe hyponatremia compared with normonatremia was 1.59 (95% CI: 1.54–1.65), 2.32 (95% CI: 2.19–2.46) and 2.40 (95% CI 2.23–2.60) respectively.
Discussion
Admission-hyponatremia was associated with increased risk of death for all levels of hyponatremia, even after adjusting for higher comorbidity level in hyponatremic patients. The risk increased with decreasing sodium levels.

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P876

Metabolic impact of IGF(CA)19 gene polymorphism on the response to GH therapy in adult GH-deficient (GHD) patients

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A highly polymorphic microsatellite in the IGF-I gene promoter composed of variable cytosine–adenine (CA) repeats (n = 10–24) has been linked to IGF-I levels, risk of diabetes and cardiovascular diseases with conflicting results. Aim of this study was to investigate the impact of this polymorphism on the response to rhGH (mean dose 0.34 $\pm$ 0.14 mg/day) in adult GH-deficient (GHD) patients after 1 (n = 98) and 5 (n = 50) years. Different genotypes were studied by microsatellite method, according to the most frequent 192 bp allele (19 CA repeats) subjects were divided into three groups: homozygous (192/192, n = 38), heterozygous (192/X, n = 44) and non-carriers (X/X, n = 16). Allelic distribution was similar in the subgroup followed for 5 years. The genotype did not influence neither the phenotype of patients at baseline (including IGF-I levels), nor their response to rhGH in terms of decrease in BF% and increase in IGF-I levels. Conversely, after 1 year, there was a significant worsening of insulin sensitivity, documented by increase in fasting glucose levels and HOMA-IR (from 81 $\pm$ 8 to 86 $\pm$ 8 mg/dl and from 1.7 $\pm$ 1.0 to 2.4 $\pm$ 1.6 respectively, $P<0.001$), as well as a significant improvement in lipid profile shown by reduction in total and LDL-cholesterol (from 215 $\pm$ 42 to 192 $\pm$ 39 and from 138 $\pm$ 38 to 111 $\pm$ 34 mg/dl respectively, $P<0.001$) only in homozygous. During long-term treatment, HOMA-IR restored to basal values in all patients, though fasting glucose levels remained higher than at baseline in homozygous. The decrease in total and LDL-cholesterol was significant both in homozygous and in heterozygous, but not in non-carriers. No difference among groups was observed in rhGH dose throughout study. In conclusion, the presence of the wild-type allele in the IGF-I gene promoter might increase sensitivity to metabolic changes induced by rhGH, either negative or positive. In the long-term, beneficial effects (i.e. persistent BF% reduction) may overcome the negative impact on glucose metabolism regardless IGF-I genotype, while positive effects on lipid profile manifest in patients carrying at least one 192 bp allele.

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P877

Hypopituitarism after fractionated stereotactic radiation therapy of anterior skull base meningiomas is caused by mass effects, not radiation

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Introduction
Fractionated stereotactic radiotherapy (FSRT) is used in the treatment of surgically inaccessible meningiomas, primarily in the base of the skull. Development of hypopituitarism following FSRT is inexhaustively investigated. We aimed to elucidate the cause of hypopituitarism in patients treated with FSRT for benign meningiomas of the anterior skull base.
Methods
All patients undergoing FSRT for benign meningioma of the skull base between July 2003 and June 2009 were included in the study. Patients baseline was established before FSRT and they were then followed with regular neuroendocrinological testing. Tumour control was monitored at neurosurgical follow-up every 2 years using MRI. Pituitary radiation dose was calculated using the dose-volume histogram for the pituitary gland generated by the radiation planning program.
Results
A 31 patients were included in the study. Median follow up was 5.2 years (range 2.2–7.8). Ten patients developed one or more new pituitary hormone deficits. Five years after FSRT, 70–79% of patients retained sufficient hormone function in each axis included in the study. Tumour control (defined as either a stable or regressing tumour) was obtained in 81% of cases. Median biological effective dose to the pituitary gland was 83 Gy (range 32–92). Multiple linear regression analysis showed that there was a statistically significant relationship between i) failure of tumour control ($P=0.0005$), ii) tumour in the pituitary sella ($P<0.0001$) and iii) time from FSRT to latest follow-up ($P=0.0116$) and the number of affected pituitary axes.
Conclusion
Our results show a significant relationship between mass effects of the tumour and pituitary axis failure as well as a significant time component in the development of hypopituitarism in these patients. It is possible that the findings of this study can explain pituitary axis failure in patients treated with radiation therapy for pituitary adenoma and craniopharyngeoma.

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P878

Role of IGF(CA)19 gene polymorphism in the clinical presentation of acromegaly

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Introduction
A highly polymorphic microsatellite, comprising a variable length of a cytosine–adenosine (CA) repeat sequence, has been identified in the promoter region of IGF-I gene. The number of CA repeats ranges between 10 and 24 and the most common allele in the Caucasian population contains 19 CA (192 bp) repeats. Several studies investigated the relationship between this polymorphism and IGF-I levels, with conflicting results. Aim of this study was to investigate the influence of this polymorphism on clinical and biochemical characteristics in 88 acromegalic patients.
Materials and methods
Different genotypes were studied by microsatellite method and patients were divided into three groups: group A, homozygous for 192 bp allele ($n=26$, 29.2%), group B, with a number of repeats ≥ 19 ($n=36$, 40%,) and group C, with a number of repeats ≤ 19 ($n=27$, 30%). Ninety-eight healthy patients were analyzed as controls.
Results
No difference in the frequency of the different alleles was observed between patients and controls. In the acromegalic population, the genotype did not influence IGF-I level at diagnosis. However, a worse of insulin sensitivity documented by a significant increase ($P=0.01$) in HOMA-IR was observed in group B (6.2 ± 5.9) compared with group A (5.0 ± 3.3) and C (4.0 ± 3.1). Moreover, higher levels of total cholesterol and LDL ($P=0.01$ and $P=0.01$ respectively) were present in group B (233 ± 49 and 168.5 ± 46.6 respectively) compared to group C (175.3 ± 43.4 and 104.0 ± 38.2 respectively). Interestingly, the number of discrepant patients (high IGF-I and normal GH levels) during medical therapy was significantly higher in group B compared to groups A ($P=0.02$) and C ($P=0.05$).
Conclusion
Different IGF-I genotypes do not account for a different presentation in acromegalic patients. Nevertheless, our data suggest that a number of CA repeat higher than 19 may be related to a worse glucidic and lipidic metabolism and to a partial disease control during medical treatment.

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Relationship between early endocrine alterations and functional outcome in patients with severe brain injury

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Introduction
Recently, it has been suggested that hypopituitarism developed after the brain injury has implications in the final recovery of the patients. We hypothesize that hypopituitarism developed in the course of traumatic brain injury (TBI) or non-TBI is related to worse functional outcome. The aim was to assess the association between hypopituitarism in the early recovery phase and long-term functional outcome.
Materials and methods
We included 157 brain-injured patients (107 TBI; 50 non-TBI) referred to the Department of Neurorehabilitation, Hvidovre Hospital from 06/2007–05/2011. Pituitary assessment including a 250 μg Synacthen® test was performed 3.5 (±1.5) months post-injury. Insufficiencies were defined as pituitary and peripheral hormone concentrations in relation to local gender and age-related reference ranges. Functional outcome was assessed by Glasgow outcome scale-extended (GOS-E), functional independence measure (FIM), and Rancho Los Amigos scale at 1-year follow-up.
Results
Hormone alterations were observed in 72.5% of the patients. Hyperprolactinaemia, secondary hypogonadism, and hypothyroidism were recorded in 50, 21, and 9% of TBI patients and in 48, 22, and 17% of non-TBI patients, respectively. Adrenal insufficiency was recorded in one non-TBI patient. Presence of secondary insufficiencies was unrelated to aetiology, shearing lesions, time since injury and antiepileptic drug treatment. Hyperprolactinaemia was related to longer posttraumatic amnesia ($P=0.02$). Hyperprolactinaemia and secondary hypogonadism were related to worse outcome at 1 year follow-up, as measured by FIM ($P<0.03$) and GOS-E ($P<0.01$). In non-TBI patients no such relations were demonstrated.
Conclusion
A substantial number of severely brain-injured patients had hormonal alterations approximately 3-months post-injury. In the TBI group, hyperprolactinaemia and secondary hypogonadism was related to poorer outcome. The importance of the observed alterations for the rehabilitation process, and the long-term consequences, however, remain to be proven.

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P880

Limitations of basal IGF-1 status reflecting the severity of growth hormone deficiency and predicting response to replacement therapy

A retrospective cohort study in a tertiary referral centre in Spain
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GH replacement (GHR) in adults has demonstrated to improve body composition, inflammatory cardiovascular biomarkers and quality of life. However, this benefit is uneven, and factors that stratify treatment response are required.
According to current guidelines, low IGF-I levels in specific conditions provide strong evidence for GH deficiency (GHD). Nevertheless, normal levels do not exclude this diagnosis and make mandatory the use of GH stimulation testing. It has not been described before if GH deficient patients with normal IGF-I get the same clinical benefit from replacement as individuals with low IGF-I.
The aim of this study is to compare the response to GHR between the subgroup with normal IGF-I and patients with low IGF-I.
We analyzed retrospectively 34 GHD adults (mean age 40.4 years; 16 females) from our centre who received GHR for at least 2 years (mean duration of treatment 7.4 years). Anthropometric parameters, bone mineral density (BMD) of the lumbar spine and hip, and the scores in quality of life questionnaires (AGHDA and Nottingham) were measured before starting treatment and at the end of the follow-up. Differences in these parameters were tested by Mann–Whitney *U* test. No significant differences were found in the baseline parameters between the subgroup with normal IGF-I and patients with low IGF-I.
The AGHDA and Nottingham mean scores improved significantly at the end of follow-up (-3 (SD 3.95) $P=0.003$ and -3.8 (SD 7.4) $P=0.01$ respectively) but there were no differences between patients with normal or low IGF-I ($P<0.109$ and $P<0.533$). No significant changes were observed in BMD or body composition.
Our data show that benefits of GHR are maintained across different scales of IGF-1 secretion. Thus, this study highlights the limitations of basal IGF-1 status in reflecting the physiopathology of GHD, indicating the severity of GHD and selecting candidates for replacement.

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P881

Effects of gender and body composition on GH response to GHRH + Arg in HIV-lipodystrophic patients: higher rate of GH deficiency in men

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Background
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)GH response to GHRH + Arg is impaired in HIV-infected men and women, compared to gender matched controls. Moreover, reduced GH secretion seems to occur more frequently in HIV-infected males than females.
Methods
To determine gender effects on GH secretion in HIV-infected patients with lipodystrophy, we compared GH/IGF1 status and body composition in 103 males and 97 females. A standardized GHRH + Arg test was performed. Anthropometric measurements were obtained by means of BMI, waist/hip, dual-energy X-ray absorptiometry (DEXA) and abdominal CT scan.
Results
Considering the threshold of GH peak of 7.5 μg/l, 21% of women and 38% of men demonstrated an impaired GH peak. Comparing males and females with insufficient GH peak, they did not differ with regard to BMI, fat mass measured by DEXA (total, at arm, at leg, at trunk) and VAT, SAT and TAT measured by CT. However, men showed higher values of VAT/SAT and VAT/TAT ratios ($P<0.05$). The intra-gender comparison showed that body composition was not significantly different between women with GH peak ≤ 7.5 and >7.5 μg/l. Conversely, men with GH deficiency had higher values of trunk fat mass at DEXA and of VAT and TAT at CT ($P<0.05$), compared to men with normal GH peak.
Conclusions
Impaired GH response to GHRH + Arg is very common in HIV-lipodystrophic subjects. Men demonstrate a higher rate of GH deficiency compared to women. Adipose tissue seems to influence GH peak in males more than in females. However, distribution of adipose tissue more than fat mass per se seems to have a role in the upset of GH/IGF1 status in these patients. Both in men and women body composition changes alone do not fully account for gender differences in GH secretory response in HIV-infected patients. Thus, an impairment of hypothalamic-pituitary function due to other factors (e.g. viral infection, antiretroviral drugs) could not be ruled out.

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P882

Obesity in patients with craniopharyngioma seems to be caused by eating disorders rather than changes in mood or activity

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Introduction
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)It is still unclear whether obesity in craniopharyngioma (CP) is caused by disorders in food regulation or by changes in mood or activity due to depression or sleepiness leading to a decrease in energy consumption. We compared CP to patients with non-functioning pituitary adenoma (NFPA) by using standardized questionnaires to clarify this question.
Methods
We compared 31 CP (m = 14, f = 17, median age: 53 years (26–77)) to 26 NFPA (m = 19, f = 7, median age: 65 years (44–80)). Patients were asked to complete eleven standardized German questionnaires. Two questionnaires evaluated eating disorders (FEV, Eating-Disorder-Examination-Questionnaire), one depression (BDI, Beck-Depression-Inventory), one anxiety (STAI, State-Trait Anxiety Inventory), three health-related quality of life (SF-36, EuroQoL, Qol-AGHDA), two sleepiness (Epworth Sleepiness Scale), two personality (EPQ-RK, Eysenck Personality Questionnaire-Revised; TPQ, Tridimensional Personality Questionnaire) and one body image (FKB-20).
Results
Both groups had the same prevalence of hormonal insufficiencies of the anterior pituitary. Patients with CP scored significantly higher in concious hunger perception (FEV, CP 6 scores (1–12), NFPA 2 scores (0–11), $P=0.016$) and in the Eating-Disorder-Examination-Questionnaire (CP 1.7 scores (0.1–3.7), NFPA 0.8 scores (0–4.4), $P=0.039$). In the latter the score for the subscale eating concern were higher compared to NFPA (CP 2 scores (0–5), NFPA 0.8 scores (0–6), $P=0.04$). Furthermore, they had higher scores in anxiety (STAI, CP 39 scores (23–60), NFPA 32 scores (23–62), $P=0.052$) and disturbed eating behaviour (FEV, CP 5 scores (0–12), NFPA 3 scores (0–13), $P=0.064$), but without reaching statistical significance. No differences could be seen in depression, personality, quality of life or sleepiness.
Conclusion
CP score higher in questionnaires of eating disorders than NFPA but not in questionnaires concerning mood or activity. Therefore, obesity in CP appears to be a consequence of eating disorders.
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P883

Correlation of clinical smell test and magnetic resonance imaging of olfactory system in idiopathig hypogonadotropic hypogonadism

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Objectives
i) To measure olfactory bulbs and sulci using dedicated magnetic resonance imaging (MRI) sequences in idiopathic isolated hypogonadotropic hypogonadism (IHH) patients with a well detailed phenotype characterization. ii) To correlate MRI findings with a clinical smell test.

Methods
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)MRI was performed in 20 patients (all males, aged between 11 and 45 years, mean age of 26) with IHH and olfactory dysfunction was assessed using the smell identification test (UPSIT), a qualitative suprathreshold olfaction test obtained from the Universityof Pennsylvania. Coronal spin echo T2-weighted and volumetric T1-weighted gradient echo sequences were acquired in a 1.5T system. ImageJ software was used to obtain olfactory bulb volumes and olfactory sulcus depths and lengths. Data were analyzed with SPSS 15.0 and the Kappa index was used to evaluate the agreement between the UPSIT and MRI.
Results
The UPSIT revealed normosmia, hyposmia and anosmia in 10 (50%), 4 (20%) and 6 (30%) patients respectively. Fourteen patients (70%) had olfactory abnormalities in the MRI. Commonest abnormality was hypoplasia seen in 8 patients (40%). Five patients (25%) had olfactory bulb. One patient had unilateral hypoplasia with normal sense of smell. There was moderate agreement between the MRI quantitative evaluation and the UPSIT (overall Kappa $=0.55$).
Discussion
Olfactory bulb and sulcus aplasia were the most common findings in IHH patients (70%). We objectively demonstrated agreement between MRI findings and the smell test, especially the presence of bulb aplasia and anosmia, confirming the high specificity of MRI findings.
Conclusion
Therefore, our findings help ascertain MRI accuracy in the diagnosis of IHH, differentiating patients with hypogonadotropic hypogonadism with an apparently normal or difficult to evaluate sense of smell.

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P884

Diencepephalic syndrome before diagnosis of childhood craniopharyngioma: results of multinational studies on 485 long-term survivors after childhood craniopharyngioma

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Background
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)Hypothalamic involvement (HI) resulting in severe obesity is known to have major impact on quality of life in craniopharyngioma (CP) patients. HI is also associated with disturbances of satiety regulation leading to a failure to thrive and weight loss known as diencephalic syndrome (DS). The rate of DS and the outcome of CP patients with DS is unknown.
Methods
CP patients have been recruited in HIT-ENDO and KRANIOPHARYNGEOM 2000/2007. 21 CP patients (4.3%) presented with a BMI <-2 s.d. at diagnosis. In 4 of 21 cases low BMI could be explained by prematurity or congenital heart failure. Eleven patients presented with DS due to proven hypothalamic involvement (HI). Three patients presented without HI, in three patients HI was not evaluable. We compared weight development since birth at standardized time points (based on a German health survey) in CP presenting DS, normal weight or obesity (BMI >3 s.d.) at the time of diagnosis.
Results
Weight development during early childhood could be analyzed in 9 of 11 DS patients. Decreases in BMI (>-1 s.d.) were detectable in 4 patients within the first year of life, in two patients in the second year of life, in two patients in the 5th year, one patient was already dystrophic at birth. Accordingly, 7 of 11 patients showed BMI reduction within the first two years of life. During follow-up, DS patients showed a significant postoperative weight gain comparable to patients who presented with normal weight at time of diagnosis resulting in obesity (median BMI $+3.98$ s.d.) after 8–12 years.
Conclusion
DS is a rare clinical manifestation of CP. In the majority BMI SDS reduction becomes manifest in early childhood, in some cases changes in BMI SDS develop later, but years before other symptoms are obvious. Low BMI at time of diagnosis does not prevent weight gain in CP with DS.

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P885

Xanthogranuloma, Rathke's cyst, and Childhood Craniopharyngioma: results of prospective multinational studies of children and adolescents with rare sellar malformations

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Background
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)Craniopharyngioma (CP), Rathke's cyst (RC) and xanthogranuloma (XG) are closely related rare sellar masses, which share common embryogenic origin. Treatment strategies in children lack consensus, especially in terms of surgical and radiooncological treatment options.
Objective
To study clinical manifestations and treatment-related outco-me in RC, XG, and CP patients.
Patients and methods
Multicentre surveillance trial. Inclusion criteria were: i) histological diagnosis of CP, XG, or RC; ii) diagnosis. Main Outcome: overall survival, event-free survival (OS, EFS), quality of life (QoL). 14 RC, 14 XG, and 117 CP patients were included in the study.
Results
Five-Year OS rates are 1.00 ± 0.00 in RC and XG; 0.97 ± 0.02 in CP.5-year EFS are 0.85 ± 0.10 in RC, 1.00 ± 0.00 in XG, and 0.50 ± 0.05 in CP. Surgical resection of XG results in complete remission without recurren-ce. Recurrences occur in RC (14%) and CR (59%), but can be efficiently treated by irradiation, reoperation, and/or intracystic treatment. Severe hypothalamic sequelae such as obesity and others effecting QoL are predominant in CP due to pre-surgical involvement (59%) and post-surgical lesions (44%) of posterior hypothalamic structures. Centres with lower neurosurgery patient load use more radical surgical approaches to treat CP, resulting in higher rates of obesity and reduced QoL. In spite of 46% anterior hypothalamic involvement, severe obesity is not encountered in XG.
Conclusions
Treatment of choice in XG and RC is radical surgery. In CP involving hypothalamic structures, less radical surgical approaches preserving hypothalamic integrity are recommended. Owing to frequent relapses, regular imaging during follow-up is recommended for CP and RC. Treatment of patients with sellar masses should be confined to experienced multidisciplinary teams. Due to the rareness of the diseases, international scientific collaboration (i.e. international trials) is recommended in order to achieve reliable results based on evaluation of larger cohorts.

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P886

Short-term exercise-induced GH response in athletes: differential results for runners and bikers when tested on bicycle

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Background/introduction
SUB:Pituitary – Clinical (*Generously supported by IPSEN*)We have previously shown that GH assessment during a standardized aerobic exercise of moderate intensity is a reliable test with high diagnostic accuracy in predicting severe GH deficiency (GHD) in adult individuals. However, these studies comprised comparably long exercise durations around 60 min or longer. To improve clinical applicability of exercise testing in the diagnosis of GHD a shorter test protocol would be preferable. The present study investigated the exercise-induced GH response in healthy athletes during a stepwise incremental VO_2 peak testing on a bicycle. We hypothesized that GH response would be smaller in bikers investigated on the bicycle compared with their running counterparts.
Methods
Nine endurance trained athletes volunteered to participate to this part of the study (seven males and two females), mean $\pm$ s.d. age was 33 ± 9.7 years. Participants

were divided into two subgroups according to their prevalent exercise habits (bikers or runners). VO_2 peak testing was performed fasting in the morning on an electrically braked bicycle with gradually increased workload until exhaustion. Blood samples for GH were taken immediately before and after exercise, as well as at 15, 30, and 45 min after end of exercise. After testing for normal distribution GH values were compared by *t*-test.
Results
The mean ± S.D. exercise duration was 11.0 ± 1.5 min. Mean VO_2 peak was 54.6 ± 5.83 ml/kg. For athletes mainly exercising on bike mean GH values were 1.97 ± 1.9, 2.29 ± 1.8, 3.33 ± 0.8, 2.23 ± 1.0 and 1.72 ± 1.4 ng/ml before, directly after exercise and at 15, 30, and 45 min after the test respectively. The corresponding GH values in athletes whose exercise mainly consisted of running: were 2.51 ± 3.3, 8.48 ± 7.9, 12.50 ± 7.5, 10.90 ± 6.6, and 9.82 ± 8.7 ng/ml. Peak GH was significantly lower in bikers compared with runners (3.59 ± 0.8 vs 15.69 ± 8.3, $P = 0.0244$).
Conclusion
Based on these preliminary results in athletes a short stepwise exercise test increasing to exhaustion appears a promising test opportunity to provoke pituitary GH secretion under standardized conditions. However, when investigating individuals performing regular exercise the type of activity may be of importance in the choice of the test conditions. While bicycle testing induced a strong GH response in runners it resulted in considerably lower GH stimulation in bikers. This difference may be due to habituation effects resulting in a weaker stimulus. Whether testing bikers on a treadmill will revert this effect will have to be studied in a next step. Furthermore, we will now investigate short-term exercise induced GH response in GH deficient patient as well as in matched but sedentary controls.

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P887

Gender determines ACTH recovery after experimental hypercortisolemia in older individuals

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Introduction
Available clinical literature raises the possibility that stress-responsive mechanisms differ by gender, especially in older individuals; however the accompanying evidence is both limited and discrepant. We hypothesize that gender determines the degree of ACTH inhibition by and recovery after an experimental cortisol clamp in older subjects.
Subjects and design
We conducted a prospectively randomized, double-blind, placebo-controlled, crossover study in 10 older men and 10 post-menopausal women (age group 50–80 years) in the clinical research unit of a tertiary medical center.
Methods
Volunteers received oral placebo or ketoconazole (KTCZ), to inhibit adrenal steroidogenesis, along with 8-h i.v. infusions of saline or a low (2.5 mg/m^2) vs. high (10 mg/m^2) physiological dose of i.v. cortisol. ACTH and cortisol concentrations were measured every 10 min during the last 4 h of the saline and cortisol infusions (feedback-clamp phase) and for 10 h thereafter (recovery phase).
Primary outcomes measure
Plasma ACTH concentrations.
Results
Gender did not determine mean ACTH concentrations during the feedback-clamp phase of saline or cortisol infusions. However, compared with men, women had markedly impaired mean (and peak) ACTH recovery from experimental cortisol infusion ($P = 0.005$, KTCZ/low-dose cortisol arm; $P = 0.006$, KTCZ/high-dose cortisol arm). Non-linear regression of ACTH on time confirmed attenuated ACTH recovery in women. Decreased ACTH recovery was accompanied by lower mean cortisol concentrations, pointing to attenuated feedforward drive of ACTH secretion rather than reduced cortisol clearance as the sex-related mechanism.
Conclusion
Gender determines the recovery of the hypothalamic–pituitary–ACTH unit from cortisol-induced feedback, with attenuated responses in post-menopausal women. The gender differences may have relevance to stress-related adaptations in the sexes.

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P888

Levels of prolactin, FSH and LH pool vs single sample determination

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Introduction
The assessment of pituitary function is often achieved by means of sequential determinations (pool) of hormone levels such as prolactin and gonadotrophins. For the determination of prolactin levels the guidelines of the Endocrine Society are clear in recommending a single sample determination.
Objective
To evaluate differences between single sample and pool (0′, 20′ and 60′) determinations for assessing prolactin, FSH and LH levels.
Methods
We conducted a cross-sectional study including 4610 prolactin pools, 2628 FSH pools and 2568 LH pools performed between 2009 and 2011. Statistical analysis was performed using the paired samples *t*-test.
Results
We found differences between prolactin levels in determinations at 0′, 20′ and 60′ (21.5 ± 64.6 vs 19.7 ± 63.7 vs 18.8 ± 63.8 ng/ml, $P < 0.001$) and also between the prolactin level at 0′ and the mean of the three determinations (21.5 ± 64.6 vs 20.0 ± 63.9 ng/ml, $P < 0.001$). The mean difference between prolactin levels at 0′ and 20′ was 1.8 ± 7.6 ng/ml, at 0′ and 60′ 2.8 ± 8.6 ng/ml and at 20′ and 60′ 1.0 ± 7.1 ng/ml. Regarding FSH pools there were also found differences in hormone levels between determinations at 0′, 20′ and 60′ and between the FSH level at 0′ and the mean of three determinations (20.9 ± 27.3 vs 20.1 ± 20.4 vs 10.0 ± 26.3 mUI/ml, $P < 0.001$; 20.9 ± 27.3 vs 20.3 ± 26.6 mUI/ml, $P < 0.001$), and the same was observed for LH pools (11.9 ± 12.8 vs 11.4 ± 12.3 vs 11.0 ± 12.1 mUI/ml, $P < 0.001$, 11.9 ± 12.8 vs 11.4 ± 12.3 mUI/ml, $P < 0.001$). The mean difference between the FSH levels at 0′ and 20′ was 0.7 ± 2.3 mUI/ml, at 0′ and 60′ 0.9 ± 2.3 mUI/ml and at 20′ and 60′ 0.1 ± 1.9 mUI/ml when considering LH pools the results were 0.5 ± 2.2, 1.0 ± 2.5, and 0.4 ± 1.8 mUI/ml.
Conclusion
Despite the presence of differences in hormone levels between pool determinations these differences, given its range, do not appear to have clinical significance.

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P889

Tumors with simultaneous hypersecretion of somatotropin and prolactin are associated with earlier diagnosis compared with tumors with isolated hysecretion of somatotropin

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Introduction
Amenorrhoea and galactorrhoea are manifestations that may allow earlier diagnosis of pituitary tumors associated with excess somatotropin (ST) and prolactin (PRL) levels.
Objective
To evaluate clinical, analytical and imaging characteristics of ST and PRL producing tumors and its affect on diagnosis.
Methods
Retrospective study including acromegalic patients diagnosed between 1982 and 2012. Information on clinical, analytical and imaging parameters was collected. For statistical analysis independent samples *t*-test, Mann–Whitney and χ^2 tests as well as partial correlations were used.
Results
We evaluated 98 patients (69.4% women, $n = 68$) with mean age at diagnosis of 45.4 ± 14.6 years and diagnostic delay of 6.8 ± 5.3 years. We compared patients with isolated hypersecretion of ST and patients with concomitant hypersecretion of PRL and ST (36.7%, $n = 36$; PRL levels of 10.8 ± 5.4 vs 78.0 ± 80.1 ng/ml; $P < 0.001$). Patients with PRL secretion showed earlier diagnosis (5.1 ± 4.1 vs 7.6 ± 5.8 years, $P = 0.021$) and were younger at diagnosis (40.3 ± 14.9 vs 48.7 ± 14.0 years, $P = 0.009$). No correlation was found between the delay in diagnosis

and prolactin levels after controlling for age at diagnosis ($r=-0.085$, $P=0.454$). There were no differences in body mass index (28.4 ± 4.8 vs 28.9 ± 6.1 kg/m^2, $P=0.678$), tumor size (19.3 ± 13.4 vs 15.2 ± 7.8 mm, $P=0.328$), ST nadir (19.8 ± 25.5 vs 19.5 ± 21.3 ng/ml, $P=0.959$), IGF1 levels (expressed as a percentage of the upper limit of normal), FSH or LH. No differences were found regarding clinical manifestations or symptoms. When evaluated independently, women and men, we found a higher prevalence of galactorrhea in women with PRL-producing tumors (14.3 vs 4.1%, $P=0.001$). When evaluated only premenopausal women ($n=19$) we found no differences between the two groups except for the levels of PRL.

Discussion

Tumors with hypersecretion ST and PRL are associated with earlier diagnosis comparing with tumors producing only ST. The shortest time to diagnosis seems especially related to age at diagnosis and not with PRL levels.

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P890

Macroprolactinomas: dopamine agonists for how long?

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Introduction

Dopamine agonists (DA) effectively normalize prolactin secretion and reduce tumour size in most patients with macroprolactinomas. However, some patients are considered partially/totally resistant. Some authors propose that patients treated for 2 years, with normal prolactin secretion with low dose AD and maximal tumour diameter reduction $>50\%$, could suspend treatment, but relapse rate is uncertain. Definition of resistance, ideal duration of treatment and criteria to evaluate reduction of tumour size have to be clarified.

Objectives and methods

Observational, analytical and retrospective study to assess the response of macroprolactinomas to DA in the first 2 years of treatment and define three groups of response based on prolactin levels and maximal tumour diameter reduction: resistant (high PRL, $<10\%$); sensitive (normal PRL $>50\%$) and partially resistant (PR) the remaining, and analyze their outcome.

Results

Fifty-two patients, 51.9% males; mean age at diagnosis 40.3 ± 16.3 years; mean follow-up 6.8 ± 4.1 years; 90.4% treated with bromocriptine, initial median dose 5.0 mg/day (P25–3.75 mg; P75-7.5 mg). After 2 years, 48% sensitive; 41.9% PR; 9.3% resistant. After 1-year, sensitive and PR patients normalized PRL in 85 and 50% and reduced tumour size $>50\%$ in 50 and 5.9% respectively. In both groups, PRL normalization didn't improve significantly in the second year. This period was important for imagiologic response in sensitive ($P=0.003$), but not in PR ($P=0.083$). In resistant, there wasn't significant improvement in either parameter after 2 years or afterwards ($P=0.331$). At follow-up, 19% of sensitive had withdrawn treatment, without recurrence. Although PR patients improved prolactin normalization (86.7%; $P=0.016$) and tumour reduction ($>50\%$:53.3%; $P=0.008$) at follow-up, they still had a less favourable outcome.

Conclusion

In our series, biochemical response preceded imagiologic response. Therapeutic results after 2 years were already present at 1 year of treatment and were maintained afterwards. PR had a less favourable outcome at follow-up, making their identification important for earlier change of therapeutic approach.

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P891

Role of reversal of hypogonadism on the improvement of metabolic syndrome in male patients with hyperprolactinemia during chronic treatment with cabergoline

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Introduction

Hyperprolactinemia (HPRL) is associated with an impaired metabolic profile, particularly in patients with hypogonadism. This study aimed to: i) investigate the effects of short and long treatment with cabergoline (CAB) on the prevalence of metabolic syndrome (MS) and insulin sensitivity and ii) to evaluate the impact of gonadal function on metabolic changes, in male patients with HPRL.

Patients and methods

Nineteen patients (41 ± 13.9 years) with HPRL entered the study. PRL, total testosterone, BMI, waist circumference, lipid and glucose profile and fasting insulin levels were assessed at diagnosis and after short (12 months) and long-term (60 months) treatment with CAB. NCEP-ATP III criteria were used.

Results

At baseline, the prevalence of hypogonadism was 73% while prevalence of MS was 52%: MS prevalence was higher in patients with (74%) than in those without (25%) hypogonadism. A significantly higher waist and BMI was found in hypogonadic than in normogonadic patients. CAB induced PRL normalization in 74 and 92% of patients after 12 and 60 months respectively. Hypogonadism was found in 42 and 10% after respectively 12 and 60 months, where MS significantly decreased to 10%. Serum triglycerides, total cholesterol, insulin levels, BMI and HOMA-IR were significantly reduced after 12 and 60 months whereas an increase of HDL was registered after 60-month follow-up. CAB dose and PRL changes correlated with the changes in BMI after long-term treatment. Testosterone levels negatively correlated with waist, insulin levels and HOMA-IR either at baseline and after treatment. Testosterone levels was the best predictor of HOMA-IR.

Conclusions

In conclusion, normal testosterone levels play an important role in the amelioration of MS of patients with HPRL by improving of insulin resistance.

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P892

Measurement of hGH in acromegalic patients under pegvisomant treatment

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Treatment with hormone analogues can challenge the precise measurement by interference with the routinely used immunoassays systems, related to the presence of autoantibodies in the serum, interference with associated therapy or the presence of altered forms of the hormone, with modified biological activity and/or immunoreactivity.

In patients with acromegaly treated with the hGH antagonist Pegvisomant (PEG; Somavert), most of the assays are deeply influenced. The current recommendation for assessing secretion status in this setting is by basal IGF1 assays.

In our study, we performed hGH measurement during OGTT or in a four-points day curve. Six acromegalic patients, four of them treated with 10–30 mg PEG s.c. daily, and two without PEG were evaluated using the following methods:

i) ^{125}I-IRMA HGH MAIA Clone (ADALTIS Italy), a liquid phase reaction immunoassay with magnetic separation of antibody–antigen–^{125}I antibody complex, not corrected for B2036-PEG cross-reactivity ($n=33$ samples).

ii) ^{125}I-IRMA HGH CT (ADALTIS Italy), a two-site immunoassay, with a solid phase reaction on tubes coated with antibody–antigen–^{125}I antibody complex ($n=50$ samples).

In patients without PEG treatment, the correlation between GH values with the two assays was 0.91 and 0.99. In patients treated with PEG, all values obtained with the first method were under the detection limit (<0.5 ng/ml). GH levels obtained with the second method were related with the tumor status of each patient, in four out of seven set of data being in a similar range to GH levels before PEG treatment, in two lower and in one higher ($P<0.05$). It seems that the second assay does not interfere with B2036-PEG, possibly due to the fact that in this method serum GH, but not PEG, binds directly to the anti-GH antibody that is coated on the tube.

In conclusion, hGH assays in acromegalic patients treated with Pegvisomant must be carefully checked for being appropriate for this specific clinical setting.

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P893

Myeloproliferative neoplasms in patients with acromegaly

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Introduction
The coexistence of acromegaly and secondary polycythemia has been described in a few patients. This association was ascribed to GH/IGF1 proliferative effect on bone marrow progenitor cells. In contrast, the presence of myeloproliferative neoplasm (MPN) with documented JAK2 mutation so far has been described in only one acromegalic patient.
Methods
We analyzed the prevalence of MPN in patients with pituitary acromegaly in a retrospective study conducted on 100 consecutive acromegalic patients, admitted to the Centre between January 2003 and January 2013. Control group consisted of 408 consecutive patients with diabetes mellitus type 2 (DMT2) matched for age and sex, admitted in the same period.
Results
Three of 100 (3%) acromegalics and none of the patients with DMT2 had MPN ($P<0.01$). One patient was diagnosed with polycythemia vera (PV), one with essential thrombocythemia (ET) and one with primary myelofibrosis (MF). JAK2 V617F mutation was found in patients with PV and ET. In addition to pituitary tumor, patient with PV was diagnosed with primary hyperparathyroidism, but no mutation was found in MEN1 gene. Due to persistent hypersomatotropism after pituitary surgery, all patients with MPN were treated with somatostatin analogues. Carriers of JAK2 mutation required treatment with hydroxyurea.
Conclusion
These preliminary results suggest that patients with acromegaly might be at increased risk for development of MPN. Whether the excessive GH/IGF1 axis activity has promotive role in the pathogenesis of MPN needs to be further investigated.

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P894

Familiar panhypopituitarism by a mutation in PROP1: four of seven brothers affected

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Introduction
PROP1 (Prophet of Pit-1) mutations are the most frequent genetic cause of panhypopituitarism, a condition associated with a deficiency or inadequate production of hormones of the anterior pituitary. The PROP1 gene encodes a transcription factor involved in the ontogeny, differentiation and function of somatotrophs, lactotrophs and thyrotrophs. These mutations are characterized by a remarkable clinical variability, including time of onset of hormonal deficiencies, hypophyseal dimensions and secretion of cortisol.
Case report
We describe a family of consanguineous parents (second-degree cousins), composed of eight siblings, four with panhypopituitarism, followed in department of Endocrinology, three healthy, and one stillbirth. Two brothers, 41 and 45 years of age, had an initial diagnosis of dwarfism at 9 and 12 years old respectively. Subsequently, it was detected TSH, FSH/LH and prolactin deficiency, in both. In the latter it was also diagnosed cortisol deficiency. The two sisters, aged 46 and 50 years old, had the diagnosis of panhypopituitarism with deficiency of GH, TSH, FSH/LH, prolactin and cortisol, since 15 and 9 years old respectively. There was no previous family history of panhypopituitarism. The genetic study was performed in the four brothers, detecting a homozygous mutation in the PROP1 gene (c.301-302delAG).
Conclusion
This case reflects the variability of clinical expression and the progressive functional impairment, including pituitary secretion of cortisol, in patients with PROP1 gene mutations.

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P895

Cushing's disease in children – the effectiveness and complications of transsphenoidal surgery

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Introduction
ACTH-secreting pituitary adenomas are the most common cause of endogenous hypercortisolemia in children after 10 years of age. In spite of this pediatric Cushing's disease (pCD) is a rare medical condition. Transsphenoidal surgery (TSS) remains the treatment of choice but – according to literature date – results of such a procedure could be worse comparatively to adult population. The aim of the study was to evaluate the clinical course, safety, efficacy and complications of TSS for pCD.
Method
Among 312 patients with Cushing's disease operated on between 2000 and 2011 we identified 11 patients with pCD – five boys and six girls. The mean age was 16.9 years (range: 13–18). The diagnosis was based on commonly adopted hormonal criteria as well as preoperative magnetic resonance imaging. TSS was performed according to the same microsurgical protocol. The remission was assessed based on the early postoperative, subnormal serum cortisol levels (≤ 2.5) and its dynamics during at least 12 months follow-up.
Results
Growth retardation and overweight or obesity were present in all 11 patients. Hypertension and mental disorders were diagnosed in five of them (45.5%). In all cases, a pituitary microadenoma was precisely visualized in MRI. Based on adopted hormonal criteria nine children (81.8%) were considered to be surgically cured whereas in two patients (18.2%) the persistent CD was confirmed. There were no medical and fatal complications. Transient diabetes insipidus appeared in three patients (27.3%) and syndrome of inappropriate antydiuresis (SIAD) in additional two cases (18.2%). Pituitary insufficiency was observed in one patient (9.1%). There was no case of postoperative cerebral spinal fluid leakage or meningitis.
Conclusions
Clinical manifestation and gender distribution of pCD is different comparatively to adult population. In spite of this TSS is a safe and effective treatment leading to a high rate of biochemical remission, similar to observed in adults.

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P896

Effect of treatment outcome on co-morbidities of Cushing's disease

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Endogenous hypercortisolism leads to increased morbidity and mortality. As Cushing's disease (CD) is a rare disorder, data on co-morbidities outcome after current treatment interventions are scarce.
We studied 78 patients with CD (67 females, 11 males, mean age 42.2 ± 1.4 years), with a follow up of 90.8 ± 7.8 months. All patients underwent transsphenoidal surgery (TSS). The criterion for successful outcome was a post-operative morning cortisol <2 μg/dl followed by sustained normalization of cortisol secretion based on midnight, urinary and post-dexamethasone corisol levels. Patients who did not meet this criterion were submitted in a combination of second line treatments (medications, pituitary irradiation, and bilateral adrenalectomy) and were considered controlled if they achieved normal urinary cortisol and/or a mean serum cortisol level of <10 μg/dl.
Depending on treatment outcome patients were divided as follows: group A ($n=18$) patients successfully treated with TSS with normal pituitary function onwards, group B ($n=11$) patients successfully treated by TSS but with pituitary deficiencies, and group C ($n=49$) with persistence or recurrence of hypercortisolism under second line treatments. In the latter group 38 patients (group C1) were controlled while 11 patients were not (group C2).
The three groups did not differ in age, BMI, urinary and midnight cortisol levels at presentation.
A significant decline in the incidence of hypertension was noted in group A (from 44.4 to 11.1%, $P=0.004$) and in group B (from 81.8 to 18.2%, $p=0.01$). In group C a similar decline was noted only in group C1 (from 63.2 to 21.1%, $P=0.03$) but not in group C2. In all groups no statistical differences in the incidence of diabetes, osteoporosis/osteopenia and dyslipidemia were observed.
In conclusion, treatment of hypercortisolism leads to correction of hypertension, a well known mortality risk factor in CD. This improvement was observed in all controlled patients independently of the applied therapeutic interventions.

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P897

Serum GH but not IGF1 levels correlate with body composition parameters in both men and women with active acromegaly

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Introduction
Active acromegaly is reported to be associated with considerable body composition changes, with significant lean body mass changes found only in men.
Patients and methods
In patients with active acromegaly ($n=40$) 24 females and 16 males, 48.9±6.4 years old, with BMI of 28.04±1.27 kg/m^2, diagnosed 4.15±1.09 years ago, body composition was analyzed by dual-energy X-ray absorptiometry (DEXA) Hologic-W-QDR. Total body fat mass (FM), total body lean mass (LM), total body fat percent (Fat%), abdominal fat mass (abdFM), abdominal lean mass (abdLM) and abdominal fat percent (abdFat%) were correlated to serum GH and IGF1 values. Gonadal status and replacement therapy were assessed in all patients.
Results
Serum GH in men (30.3±9.4 mIU/l) correlated significantly negatively with Fat% (19.9±6.6%) ($R=-0.65$ $P<0.05$). Serum GH in females (49.2±18.1 mIU/l) correlated significantly positively with LM (48.03±7.3 kg, $R=+0.48$, $P<0.05$). Serum IGF1 did not correlate significantly with any of the body composition parameters in either gender. Smaller number of included unreplaced hypogonadal male acromegalics ($n=4$) did not differ in any of the body composition parameters compared to eugonadal male acromegalics ($n=9$) or hypogonadal patients on testosterone ($n=3$). Hypogonadal and menopausal women ($n=19$) exhibited significantly elevated FM (23.1±2.2 vs 19.3±2.1 kg, $P<0.01$), abdFM (2.8±0.4 vs 2.3±0.3 kg, $P<0.01$) compared to the smaller number of eugonadal female acromegalics ($n=5$).
Conclusion
Important body composition parameters (lean body mass, total body fat percent) correlate with GH levels but not with IGF1 in both men and women with active acromegaly. Rutine serum GH level assessment should be added to serum IGF1 determination in the assessment of the acromegaly activity. Gonadal status appears to be of greater confounding influence on body composition in female than in male active acromegalics.

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P898

Receptor expression in craniopharyngiomas causing tumor growth in pregnancy: case report and review of the literature

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Introduction
Craniopharyngiomas (CP) are benign tumors that arise from remnants of the Rathke's pouch. Classically, they are classified as cystic or calcified tumors. The presence of hormonal receptors in CP has been reported in *in vitro* studies but only eight cases growing during pregnancy have been published in literature.
Case report
We report a 32-year-old woman who was initially diagnosed with a prolactin secreting tumor with rapid enlargement during pregnancy that eventually was found to be an aggressive CP. Pregnancy was achieved with IVF. The patient had a medical history of hyperprolactinemia treated with cabergoline and a pituitary tumor of 9 mm. She consulted at 27 weeks of gestation because of visual impairment. A pituitary MRI showed a cystic pituitary tumor of 22 mm in maximum diameter with suprasellar extension and compression of the optic apparatus. A second 8 mm tumor was described in the floor of the fourth ventricle. Visual assessment had demonstrated no deficit before pregnancy. By the 33rd week of gestation the subject had right lateral homonymous hemianopsia with a decreasing visual acuity that resulted in blindness of the left eye and diabetes insipidus; a caesarean section was performed at that time. The newborn was healthy. Neurosurgery was performed postpartum by a sub-frontal approach. The pathology confirmed an adamantinomatous type CP. The second 8 mm tumor was an ependymoma grade 2.
Immunohistological staining demonstrated epithelial growth factor receptors in 100%, progesterone receptors in 10% and estrogen receptors in 5% of tumor cells. Ki-67 was <1%.
Conclusion
IVF allows pregnancy in infertile population. Only eight cases of CP growing during pregnancy have been reported. We have demonstrated expression of hormonal receptors that may explain the aggressive behaviour of CP during pregnancy. This is the only report with an associated ependymoma.

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P899

Severe hyponatraemia due to SIADH is associated with very high 5-year mortality

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Purpose/background
SIADH is a common cause of severe hyponatraemia in hospital and is due to a variety of underlying aetiologies. Kidney injury (KI) which may be associated with severe hyponatraemia is associated with poor clinical outcomes. It is unclear whether severe hyponatraemia due to SIADH is also linked to adverse outcomes. We therefore compared the 5-year outcome (mortality) in patients with severe hyponatraemia (Na≤125 mmol/l) caused by SIADH with those due to kidney injury.
Methods
Retrospective observational cohort analyses of anonymised data over a 3-year period. All patients had a mean Na ≤125 mmol/l. Additional criteria used were –KI: urea >7.5 mmol/l, creatinine >130 μmol/l and SIADH: urea <3.6 mmol/l, creatinine <130 μmol/l. Mortality data was reviewed up to 5 years from the date of the last test and was right-censored at 5 years.
Results
Mean (S.D.) serum sodium levels were 121.5 (±4.0) and 122.1 (±3.0) mmol/l in those with SIADH ($n=326$) and KI ($n=313$) respectively. The corresponding values of serum urea were 2.6 (0.7) and 22.0 (11.1) mmol/l. 73.4% of patients with KI died within the first year after admission; the corresponding figure for SIADH patients was 56.0%. At 5-year the corresponding mortality figures were 86.7% (KI) and 79.0% (SIADH). Kaplan-Meir curves showed a statistically significant (log-rank, $P<0.0001$) shorter median survival time for patients with KI (1.19 months (95% CI 0.70, 1.67)) compared to those with SIADH (22.8 months (95% CI 14.84, 30.73)). This difference in survival was maintained with data standardised for age at death.
Conclusions
SIADH is associated with a high first year mortality after discharge albeit less than those with KI. At 5-years the mortality rates appear to converge. This interesting observation may suggest a role of V-2 receptor antagonists in improving medium-to-long-term outcomes in some patients with SIADH.

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P900

Increased thyroid cancer risk in acromegaly

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Introduction
Acromegaly increases cancer risk. We aimed to determine the prevalence and the predictors of tumors in acromegalic patients treated at our department.
Design
We retrospectively evaluated 142 acromegalic patients (75 females (mean age 52.1±10.4 years and 67 males (mean age 49.4±12.7 years)) treated at university center, endocrinology outpatient clinic between 1990 and 2012, with a mean follow up period of 7.5±5.9 years. The patients were screened with colonoscopy, mammography, thyroid and prostate ultrasonography.
Results
Malignancy was found in 31 (21.8%) patients. No significant difference was observed in the distribution of malignancy among sexes (20.0% in females vs 23.9% in males). Thyroid cancer was the most frequent ($n=15$, 10.6%) followed by the breast cancer ($n=4$, 2.8%) and colorectal cancer ($n=4$, 2.8%). Renal cell cancer in two patients, oddi tumor in one, rectal carcinoid tumor in one, bladder cancer in one, malignant melanoma in one, prostate cancer in one, lung cancer in one, parotid mucoepidermoid carcinoma in one and malign mesenchymal tumor in brain in one patient were detected. One patient had both thyroid and renal cell cancer. The patients with cancer were significantly older than the patients without cancer and age of patients at diagnosis of acromegaly was significantly higher in patients with cancer (45.8±9.9 vs 40.7±11.6 years, $P<0.05$). No significant difference was found in duration of the disease, initial GH levels, the prevalence of diabetes, hypertension, coronary heart disease, hyperlipidemia and treatment modalities between the patients with/without cancer.
Conclusion
The risk of cancer in acromegaly especially the thyroid cancer risk seems to be more increased than known in the literature. Therefore, acromegaly patients should be screened routinely for cancer, especially for thyroid cancer due to it being up to three times higher prevalence than breast and colorectal cancer.

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P901

Efficacy and safety of lanreotide in combination with pegvisomant in clinical practice in patients with active acromegaly with monotherapy failure

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Introduction

ACROCOMB is a retrospective Spanish Multicenter study, designed to evaluate the efficacy (extent of tumour control) and safety of lanreotide (LAN) treatment combined with pegvisomant (PEG) or cabergoline in acromegalic patients with monotherapy failure.

Methods

patients with acromegaly treated with LAN+PEG (45% of ACROCOMB patients) at 44 Spanish Endocrinology Departments were analysed.

Results

40% of patients were male, median age: 42.5 years. Mean time from diagnosis was 7.2±7.5 years. Tumour size at diagnosis was 25.5±9.9 mm. 92% of patients had surgery and 65% had radiotherapy. Immediately prior to LAN+PEG, 57% were receiving LAN, 4% octreotide, 35% PEG, 2% cabergoline and one patient was not receiving treatment. Median baseline IGF1 was 156% ULN (15–534%). LAN+PEG was indicated for monotherapy failure (85%), tumoural volume control (12%), headache (8%). 14% of patients received LAN+PEG for >1 reason. Median LAN+PEG treatment duration was 2.1 years (0.4–6.3). Median monthly LAN doses were similar at baseline (120 mg (60–240)) and at end of study (EOS) (120 mg (30–240)). At baseline 21% of patients were receiving an extended LAN regimen (q6w or q8w) and at EOS 25%. Median weekly PEG doses increased from 70 mg (10–210) at baseline to 105 mg (30–210) at EOS. Median IGF1 values decreased by 6 months (83% ULN (11–236%), $P<0.0001$) and remained stable at EOS (84% ULN (23–345%), $P<0.0001$). At EOS 71% of patients had normal IGF1 values; drug infradosing might explain lack of normalization in the other patients. Tumour size decreased in 3 patients. No changes in hepatic, cardiac, glycemic parameters were reported. 41 (79%) patients continue receiving LAN+PEG at EOS.

Conclusion

The combination of LAN and PEG is well-tolerated and has high efficacy in clinical practice in patients not well controlled with monotherapy. No significant liver enzyme elevations were observed.

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P902

Efficacy and safety of lanreotide in combination with cabergoline in clinical practice in patients with active acromegaly with monotherapy failure

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Introduction

ACROCOMB, a retrospective Spanish Multicenter study, evaluated the efficacy and safety of lanreotide (LAN) combined with cabergoline (CAB), or pegvisomant in patients with acromegaly.

Methods

patients treated with LAN+CAB at 44 Spanish Endocrinology Departments were included.

Results

33% male patients, median age: 50.4 years. Mean time from diagnosis: 5.9±6.9 years. Tumour size at diagnosis: 21.9 mm. 83% of patients had undergone surgery and 38% had received radiotherapy. Medical treatment immediately prior to LAN+CAB, was LAN (57%), octreotide (10%), or dopamine agonist (15%). 70% of patients received LAN+CAB to improve their hormonal control; 17% had mixed tumours (PRL/GH) and LAN+CAB was their first medical treatment. Median LAN+CAB treatment duration was 1.5 years (0.1–6). Median LAN doses were 90 mg/month (60–120) at baseline (42% received <120 mg) and 120 mg/month (60–240) at end of study (EOS) although 22% received <120 mg. An extended LAN regimen (q6w or q8w) was administered to 13% of patients at baseline and 14% at EOS. Median CAB dose was 1 mg/week (0.25–5) at baseline and 1.4 mg/week (0.25–5) at EOS. Median GH and IGF1 values at baseline, 6 months and EOS were: GH, 4 ng/ml (0–400), 3 ng/ml (0–135), 2 ng/ml (0–103); IGF1, 144% ULN (15–505%), 115% ULN (13–557%), 105% ULN (13–557%) $P<0.0001$. At EOS, GH <2.5 ng/ml was reached in 51% of patients and normal age-adjusted IGF1 in 46%. Tumour size decreased in 8 patients. At EOS 48 (75%) patients were receiving LAN+CAB. Main reason for discontinuation was lack of efficacy (13%).

Conclusion

The LAN+CAB combination is frequently used and well-tolerated in clinical practice with efficacy similar to what has been reported in the literature, albeit a lower CAB dose, and appears to be clinically useful in some patients not controlled on monotherapy

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P903

Efficacy of hGH treatment in pituitary dwarfism- age does matter

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Introduction

The spectrum of GH deficiency (GHD) in children ranges from complete deficiency, with severe growth retardation, to partial deficiency, with slightly short stature. The administration of GH to children with GHD improves linear growth, mainly during the first 2 years of treatment.

Patients and methods

The study analyzes pattern of growth in 35 children (24 boys, 11 girls) with GHD, in the first year of treatment with hGH. There were three groups, according to pubertal stage (Tanner): ≤8 years (prepubertal) – one girls (G) and one boy (B), 8–12 years (PIIBII G, PIIGII B) – 2G, 2B and ≥12 years (minimum PIIIBIII G, PIIIGIII B) – 3G, 3B.

Results

Before treatment, SDS for height was −2.5. All had GHD biological confirmed and delayed bone age. The analysis showed: 1B-average growth rate 0.89 cm/month in the first 6 months, similar in the next 6 (0.83 cm/month), mean height gain 9.72 cm±2.6 and bone age (BA) improved with 13.1 months; 1G-average rate higher in the first 6 months (1.02 cm/month), compared to the next 6 (0.67 cm/month), mean gain 10.2 cm±2.3 BA improved with 18.5 months; 2B-average rate 0.63 cm/month in the first 6 months, similar in the next 6 (0.61 cm/month), mean gain 7.33 cm±0.77 BA improved with 17.5 months; 2G-average rate 0.63 cm/month in the first 6 months, 0.78 cm/month in the next 6, mean gain 8.55 cm±1.63 BA improved with 9 months; 3B-average rate 0.72 cm/month in the first 6 months, 0.63 cm/month in the next 6, mean gain 7.94 cm±0.83 BA improved with 15.4 months; 3G-one patient with growth rate 0.6 cm/month in the first 6 months, similar in the next 6, height gain 7.3 cm BA improved with 24 months.

Discussions
We noticed a negative correlation between growth rate and age of beginning treatment. Despite hGH dose adjustment, the growth rate remains lower in children diagnosed later, confirming the importance of an early diagnosis and treatment, prepubertal.

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P904

Clinical, hormonal and radiological characteristics of a group of primary empty sella patients

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Background
Primary empty sella is an anatomical term defining the herniation of subarachnoid space within the sella turcica without an underlying factor such as pituitary tumor, surgery, irradiation or trauma.
Patients and methods
We retrospectively evaluated clinical, hormonal and radiological data of the patients with primary empty sella syndrome followed in our institution between 2001 and 2012.
Results
Ninety-four patients (80 (85.1%) females) were included in the study. The mean age of the patients at diagnosis was 50.3 ± 15 years (15–85). Fatigue and headache were the most common presenting symptoms (47.9 and 42.6% respectively). Menstrual irregularity in women (25%) and impotence in men (35%) were the most common sex specific symptoms. In addition galactorrhea, visual abnormalities, loss of libido were also recorded at a lesser extent. Of the risk factors of primary empty sella, multiparity was detected in 80% of the female patients and hypertension in 29.8%, weight excess in 44.7%, autoimmune thyroid disorder in 12.8% of the whole study group. Patients were evaluated for pituitary function with basal hormone levels and dynamic testing when necessary At least one pituitary hormone deficiency was found in 67% of cases. Growth hormone deficiency was the most common (54.3%) hormonal abnormality. Secondary adrenal insufficiency (43.6%), hypogonadotropic hypogonadism (33%), central hypothyroidism (25.5), mild hyperprolactinemia (24.5%), and diabetes insipidus (5.3%) were also recorded. Thirty-two percent of the patients had partial and the remaining 68% total empty sella in pituitary imaging. No significant differences were found among the partial and total empty sella subgroups in terms of hormonal deficiency.
Conclusion
Primary empty sella appears to be more frequent in multiparous women and in individuals with obesity. Radiological discrimination as partial or total empty sella seems to have no effect on the degree of hypopituitarism.

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P905

The effects of cabergoline treatment on cardiac valves in patients with prolactinoma

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Introduction
Dopamine agonists have been reported to increase the risk of cardiac valve regurgitation in valve disease risks for 3 years treatment with cabergoline in patients with prolactinma. The aim of the study was to evaluate the frequency of cardiac valve diseases and regurgitation in patients with prolactinoma at the end of 3 years.
Subjects and method
Thirty-one patients with prolactinoma who had received cabergoline following 3 years were enrolled study. Two-dimensional and Doppler echocardiography were performed before treatment and after 3 years. All records were evaluated in all patients, retrospectively.
Results
Before treatment, 2 (2/31) patients had minimal mitral valve regurgitation and 1 (1/31) patient had minimal tricuspid insufficiency. Thickening of the mitral, tricuspid and aortic valves were not found before treatment. After 3 years, 1 (1/31) patients had moderate mitral valve regurgitation and 1 (1/31) patient had moderate tricuspid valve regurgitation. There were no statistically significantly differences of diameters of valves areas between two measurements (Table 1). Mean cumulative dose of cabergoline was 169.8 ± 30.2 mg. Moderate valve regurgitation was not associated with cumulative dose of cabergoline ($P = 0.3$).
Conclusions
Cabergoline therapy for 3 years was not associated with an increased risks of cardiac valve diseases and regurgitation in patients with prolactinoma.

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P906

Treatment with pasireotide LAR normalizes prolactin levels in patients with acromegaly and hyperprolactinemia: randomized, double-blind, 12-month, phase III study

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Introduction
Around 20–30% of patients with acromegaly have hyperprolactinemia, which is associated with infertility and gonadal/sexual dysfunction. Current therapy involves somatostatin analogues for GH/IGF1 excess and a dopamine agonist to decrease prolactin levels. The objectives of this analysis were to assess treatment with pasireotide LAR or octreotide LAR alone in patients with acromegaly and hyperprolactinemia.
Methods
Patients with acromegaly (GH >5 μg/l or GH nadir ≥ 1 μg/l post-OGTT, and IGF1 $>$ ULN) who were *de novo* with a visible adenoma on MRI or medically naïve (no previous medical therapy but prior pituitary surgery) received pasireotide LAR 40 mg/28 days ($n = 176$) or octreotide LAR 20 mg/28 days ($n = 182$) for 12 months; dose titration (to pasireotide LAR 20/60 mg or octreotide LAR 10/30 mg) was permitted. This analysis focuses on the efficacy/safety of pasireotide LAR and octreotide LAR in patients with baseline hyperprolactinemia (prolactin above age/sex-matched ULN).
Results
29 (16.5%) pasireotide LAR and 30 (16.5%) octreotide LAR patients had baseline hyperprolactinemia (mean prolactin 83.5 and 55.9 μg/l, respectively). After 12 months, 21/29 (72.4%; 95% CI 52.8, 87.3) pasireotide LAR and 17/30 (56.7%; 95% CI 37.4, 74.5) octreotide LAR patients had normalized prolactin levels; 10/29 (34.5%) and 13/30 (43.3%) had GH <2.5 μg/l, while 7/29 (24.1%) and 8/30 (26.7%) had normal IGF1, respectively. After 12 months, mean prolactin decreased by 60.4 and 39.6%, mean GH decreased by 71.1 and 67.6%, and mean IGF1 decreased by 41.1 and 39.6%, respectively, in pasireotide LAR and octreotide LAR patients. Tumor volume decreased by ~40% in both treatment groups. Pasireotide was well tolerated and most adverse events were mild/moderate; hyperglycemia-related adverse events were more common with pasireotide LAR than octreotide LAR.
Conclusions
In this subset of patients with baseline hyperprolactinemia, pasireotide LAR normalized prolactin in >70%, normalized IGF1 in ~25% and achieved GH <2.5 μg/l in ~35% of patients. Pasireotide may be an effective treatment for patients with a GH- and prolactin-secreting pituitary adenoma.

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P907

Characteristics and outcomes of the Italian subpopulation enrolled in the observational, multicenter hypopituitary control and complications study (HypoCCS)

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Aim
To describe characteristics and outcomes of Italian patients with hypopituitarism participating in HypoCCS.
Methods
Study population was stratified by max GH peak (mGHp) and BMI. Baseline variables included demographic characteristics, type of deficit, smoking habits; variables analyzed over time included weight, Framingham cardiovascular disease (CVD) risk, lipids, GH dose.
Results
Italian subpopulation included 342 patients with mGHp $\leq$33 (group A); 345 with 33 $<$ mGHp $\leq$ 66 (group B); and 337 with mGHpk $>$66 percentile (group C) with mean age (years (S.D.)) of 44.2 (16.2), 44. 6 (16.0), 42.6 (15.1), respectively, and adult onset GHD ((%): 75.8, 77.1 and 78.4% respectively). GHD was diagnosed mainly with GHRH + Arginine test (roughly 66% of diagnoses) and percentage of multiple pituitary hormone deficits was higher ($P<0.001$) in subgroup A (92.7) than in B (85.2) or C (69.5). Patients were equally distributed across normal-, under- and over-weight with average BMI of 28. No differences were detected in smoking habits or in Framingham CVD risk at baseline. More patients in group A than in B or C had hyperlipidemia (*n* (%): 92 (35.1), 86 (31.1), 69 (24.7) respectively; $P=0.029$). Mean GH dose at baseline was significantly lower in group A than in B and C (dose/kg (S.D.): 311.0 (162.5), 356.3 (217.9), 391.7 (323.1); $P=0.0009$) and with a longer treatment duration (years (S.D.): 7.2 (9.2), 5.5 (8.2), 5.0 (7.6); $P=0.0014$). Analyses over time showed group differences only at certain single time-points. Overall, no significant differences in treatment emergent adverse events (TEAEs) were detected across subgroups, while among the serious TEAEs, only 'infections and infestations' were significantly different (*n* (%): 6 (1.8), 5 (1.5), 0 (0.00); $P=0.0406$).
Conclusions
Italian patients with mGHp $<$33 percentile had the worst lipid profile and were given the lower GH treatment dose. The highest percentage of multiple deficits in this group suggests the more severe GHD.

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P908

Sleep-apnea and cardiomyopathy in acromegalic patients

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Introduction
Sleep disordered breathing (SDB) promotes structural myocardial changes and can trigger cardiac arrhythmias. Acromegalic patients have high prevalence of SDB and GH–IGF1 excess is related to a specific cardiomyopathy characterized by concentric cardiac hypertrophy and diastolic dysfunction. The aim of this study was to assess the relationship between SDB and cardiac dysfunction on acromegalic patients.
Material and methods
Observational descriptive study of 32 acromegalic patients (14 men, 50.3 $\pm$ 11.4 years, 21 treated with somatostatin analogues (SSA)) and 20 patients referred to the respiratory department for SDB study paired with acromegalic patients in sex, age and BMI (10 men, 53.2 $\pm$ 12.7 years). Polysomnography, echocardiography and electrocardiography (ECG) were performed in all patients. Patients were defined having sleep-apnea (SA) if they had more than ten apneas or hypopnoeas per hour. Pearson, *t*-Student and χ^2 tests were used for statistical analysis.
Results
24 (75%) acromegalic patients and 15 (78.9%) controls had SA, all of them due to obstructive cause. 18 (58.1%) acromegalic patients and 6 (30%) controls had diastolic dysfunction of left ventricle ($P=0.05$). Patients with/without SSA did not have different prevalence of SA or cardiac dysfunction. Only acromegalic patients, but not controls, with SA compared with those without SA had higher diastolic LV diameter (49.7 $\pm$ 6.9 vs 44.7 $\pm$ 3.7, $P=0.02$) and higher pulmonary artery systolic pressure (35.3 $\pm$ 4.5 vs 22.0 $\pm$ 7.6, $P=0.01$). Moreover, they had a trend toward less cardiac frequency (72.6 $\pm$ 7.7 vs 78.1 $\pm$ 9.1, $P=0.08$), less ejection fraction (62.6 $\pm$ 12.7 vs 71.4 $\pm$ 10.4, $P=0.09$) and more alterations on ECG (50 vs 12.5%, *P* 0.09).
Conclusions
SDB is a risk factor to cardiac abnormalities in acromegalic and non acromegalic people. The prevalence of cardiac abnormalities in acromegaly is higher when SA is present, independent of the cure or control of acromegaly.

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P909

PONV prophylaxis alters postoperative cortisol-measurement in transphenoidal pituitary surgery

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Objective
Postoperative nausea and vomiting is common after general anaesthesia and is reported by $\sim$20–25% of all patients. The standard prophylaxis at the authors department is a single treatment of 4 mg of dexamethasone before initiating anesthesia. Dexamethasone is known to suppress ACTH and cortisol levels. The objective was to find out whether the standard PONV-prophylaxis of 4 mg of dexamethasone has an effect on postoperative levels of cortisol in patients undergoing transsphenoidal pituitary surgery and therefore simulates pituitary defency or in cases of cushings masks persistent disease.
Methods
consecutive patients who were operated during a course of 6 months were included. 19 patients with a known history of PONV received a standard dose of 4 mg of dexamethasone perioperatively. Blood tests were drawn at the first postoperative day in the usual manner and were compared to blood tests of patients who had no history of PONV and therefore received no prophylaxis.
Results
Patients who were treated with a dexamethasone PONV-prophylaxis showed no significant changes of cortisol levels; preoperative median of 93 ng/ml, range 39–265 ng/ml postoperative 92 ng/ml, range 10–733 ng/ml ($P=0.353$) opposed to patients who did not receive such treatment; preoperative 127.5 ng/ml, range 10–387 ng/ml vs postoperative 263 ng/ml, range 10–1016 ng/ml ($P<0.001$). Paired *t*-test was used to determine significance.
Conclusions
As the early postoperative blood checks of cortisol levels yields very important information about potential pituitary insufficiency after transsphenoidal surgery, it is of crucial information that dexamethasone PONV prophylaxis suppresses (in analogy to a dexamethasone suppression test) postoperative cortisol levels in such a significant manner.

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P910

Report of a new case of the novel AIP p.R314W mutation in a Romanian sporadic acromegaly patient

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Introduction
We have recently described a novel AIP mutation c.940C $>$ T, p.R314W, in a young sporadic acromegaly patient.
Aim
To present a new case of AIP p.R314W mutation and screening results of a Romanian control group for p.R314W.
Patients and methods
One sporadic acromegaly patient, investigated by sequencing screening of all six AIP exons, following informed consent, as part of a sporadic pituitary adenoma cohort. 110 control subjects without clinical signs of pituitary adenoma (24M/86F) were screened for AIP exon 6 sequence changes using high resolution melting analysis of exon 6 PCR products.

Results
Sequencing of AIP revealed two heterozygous missense mutations in the acromegaly patient (female, 51 years old at diagnosis). The previously described c.940C>T, p.R314W was present together with a novel sequence change, c.878A>T, p.E293V. The patient presented a large somatotropinoma (30 mm) with perisellar invasion, optic chiasm syndrome, secondary diabetes mellitus, hypertension, and pituitary gonadotroph deficiency. Following initial transsphenoidal surgery a tumor remnant was present and required a second transsphenoidal intervention and pituitary radiotherapy for controlling tumor growth. Somatostatin analogues therapy could not achieve optimal control of GH secretion. p.R314W and p.E293V were absent 110 controls. *In silico* evaluation of pathogenicity (Polyphen2) of p.R314W suggested this is pathogenic, while p.E293V scored as non-pathogenic. AIP mutation screening of family members of the index case has not yet been performed.
Conclusion
We describe a novel case of AIP p.R314W mutation. This likely pathogenic mutation has been exclusively associated with sporadic acromegaly to date. Although the two reported cases are apparently unrelated, we cannot exclude a founder effect in the Romanian population. In vitro experiments are necessary for confirming the pathogenic role of this novel AIP mutation and understanding the mechanism of its deleterious effect.
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P911

The p.R16H AIP sequence variant is relatively frequent in Romanian sporadic pituitary adenoma patients

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Background
A large spectrum of AIP gene mutations has been identified in familial and sporadic pituitary adenomas (PA) with over 70 different mutations described to date. c.47G>A, p.R16H is an AIP exon 1 variant of unknown significance, its contribution to pituitary adenoma development being controversial.
Aim
Characterization of p.R16H prevalence in a large Romanian cohort of PA patients and controls.
Patients and methods
sporadic PA patients (95F/60M), mean age at symptoms onset 32 years old, were screened by sequencing of all six AIP exons and 108 control subjects (23M/85F) were screened by high resolution melting analysis of AIP exon 1 PCR products.
Results
We identified a heterozygous c.47G>A, p.R16H sequence change in four PA patients (2.58%) (1M/3F): one female acromegaly patient (35 years old at onset) with a macro-somatotropinoma resistant to somatostatin analogues, two female macroprolactinoma patients (23 and 26 years at onset) and one male nonfunctioning agressive PA (NFPA) (38 years at onset), associating familial intellectual disability.
One female control subject, 20 years old presented an abnormal AIP exon 1 PCR melting profile, confirmed by sequencing to be heterozygous p.R16H. She had been recruited while undergoing evaluation of a large thyroid nodule (FNAB diagnosis – follicular neoplasm), without clinical signs of PA (no imaging has been performed). Screening of relatives was only performed in one sister of the NFPA patient and was negative.
Conclusion
p.R16H is an AIP variant of unknown significance, relatively frequent among Romanian sporadic PA patients. More clinical and experimental data are necessary to establish the true role of this variant, in order to use this information in genetic counselling of the affected individuals.
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P912

Prevalance of cancer in acromegaly patients; a single center data

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Introduction
The growth promoting effects of GH and IGF1 has lead to increasing number of cancer surveillence studies in acromegaly so far. We herein aimed to present the prevalance of cancer in acromegaly patients followed in our institution.
Patients and design
We retrospectively analyzed medical records of 151 patients with acromegaly (83 females) followed in our department from 2001 to 2012.
Results
Overall, cancer was detected in 19 (12.6%) patients. Thyroid carcinoma was the most common cancer ($n=9$ (5.9%)) and papillary thyroid cancer was the leading type ($n=7$ (4.6%)). Besides, one patient had medullary and another one had synchronous papillary and medullary thyroid cancer. Lung cancer was found in two patients and rignet-ring-cell colon carcinoma in one, endometrium cancer in one, malignant melanoma in one and myelodysplastic syndrome in one patient. In addition, four patients had multiple endocrine neoplasia-type 1. Frequency of cancer among both sexes were not significantly different (female: 12/83 (14.4%), male: 7/68 (10.2%), $P>0.05$). Cancer was detected in 13 patients after, in four patients simultaneously and in three patients before the diagnosis of acromegaly. There were no significant differences in age of onset of acromegaly or cancer, disease duration, hormone levels and metabolic parameters among the patients with and without cancer. In logistic regression model, neither disease duration nor hormonal activity were associated with malignancy risk.
Conclusion
In parallel with most of the reports, thyroid cancer was the most common cancer in our acromegaly patient group. Although we could not demonstrate an association of disease activity with cancer, high hormone levels with possible mitogenic activity might be a causative or accelarating factor in carcinogenesis.
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P913

Comparison of insulin tolerance test and ACTH stimulation test for evaluation of hypocortisolism in patients with acromegaly

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Background
The insulin tolerance test (ITT) is considered the gold standard test for evaluating the ACTH–cortisol axis in patients with pituitary disease. However, the test requires time and personnel resources, and has clear contraindications. Therefore, an ACTH stimulation test is often performed instead.
Objective
The objective was to compare the cortisol response acquired by ACTH stimulation and during ITT in patients with pituitary disease.
Methods
In a total of eight patients with acromegaly, both ITT and an ACTH stimulation test were performed during the same week, but on separate days. A total of 20 comparisons were performed, five being preoperative and 15 postoperative.
Results
Five comparisons were excluded for analyses due to inadequate hypoglycaemia. The remaining 15 comparisons were both preoperative (three, including one after octreotide treatment) and postoperative (three at 3 months, three at 1 year and six at 5 years postoperative). During ITT, peak cortisol level was 637/745 nmol/l (mean/median). Peak cortisol after ACTH stimulation was 948/1040 nmol/l. There was significant correlation between highest plasma cortisol during ITT and ACTH stimulation (Spearman's $\rho=0.79$ and Pearson's $r=0.86$, both $P<0.001$). At ITT, four test displayed peak cortisol <550 nmol/l, of them only one ACTH stimulation test resulted in peak cortisol <550 nmol/l. The discrepant results were pre-treatment, 3 months and 5 years postoperative.
Conclusion
There is some discrepancy between cortisol response during ITT compared to ACTH stimulation test, where the latter leads to a higher peak cortisol level and less often a blunted response when using the same cut-off level.
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P914

Functional hyperprolactinemia in polycystic ovary syndrome: incidence and correlation with hormonal parameters

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Introduction
Polycystic ovary syndrome (PCOS) is associated with a complex altered hormonal profile. Functional mild hyperprolactinemia can be found in up to 25–30% of PCOS women. The aim of the study was to determine the incidence of hyperprolactinemia in PCOS patients and to correlate the prolactin values with hormonal and metabolic data.
Material and methods
The study included 25 women, diagnosed with PCOS, according to Rotterdam criteria (mean age 31.1 ± 6.3 years), evaluated in the Clinic of Endocrinology, Timisoara, Romania, in 2012. The patients with other causes of hyperprolactinemia (drugs, hypothyroidism, etc.) were excluded. The hormonal profile (prolactin, LH, FSH, testosterone, DHEA-S) was determined using chemiluminescent microparticle immunoassays. If serum prolactin level was high, to exclude macroprolactinemia, polyethyleneglycol precipitation was used.
Results
Sixteen patients presented obesity (64%). The mean prolactin value in the study group was 55.1 ± 84.5 ng/ml, median value: 20.3 ng/ml (normal values 5–17.4 ng/ml). Eleven cases (44%) presented elevated values of serum prolactin (mean 93.2 ± 104.5 ng/ml, median 39.2 ng/ml, range 20.3–210 ng/ml). The mean prolactin values in hyperprolactinemic women with amenorrhea ($n=7$) were higher (137.4 ± 78 ng/ml, median 41.2 ng/ml) than in those with present menses (87.1 ± 110 ng/ml, median 37 ng/ml, $P=0.4$). Three of the cases with mild hyperprolactinemia ($n=8$) showed normal prolactin values after polyethyleneglycol precipitation. Three patients with marked increased prolactin levels (over 100 ng/ml) performed a pituitary MRI, with normal appearance of the pituitary gland. Prolactin values did not correlate with body mass index, total testosterone, DHEA-S, LH, FSH, LH/FSH, glycemia, or lipid profile.
Conclusion
In PCOS women, functional hyperprolactinemia can occur in a significant proportion. In these cases, other causes of hyperprolactinemia must be excluded (macroprolactinemia, pituitary adenoma, hypothyroidism, drugs, etc.). The prolactin levels do not correlate with other hormonal and metabolic parameters.

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P915

Bone turnover decreases 6 months after acromegaly remission but still remains higher than in healthy population

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Introduction
The study was designed to assess the effects of remission of acromegaly on bone turnover.
Methods
Twenty-eight patients with active acromegaly (14 women; age 48.0 ± 9.8 years) were enrolled in the study. The control group comprised 28 age- and sex-matched healthy individuals. Bone turnover markers (osteocalcin, β-crosslaps, and bone alkaline phosphatase) were measured at enrolment and 6 months after achievement of disease remission. Transspehenoidal surgery was performed in all patients. Twenty-one patients achieved disease remission after surgery. In seven patients disease remission was achieved using somatostatin receptor analogs plus radiosurgery in two of them.
Results
Patients with active acromegaly had significantly higher levels of osteocalcin ($P=0.003$), β-crosslaps ($P<0.001$) and bone alkaline phospahatase ($P<0.001$) than the control group. Six months after remission achievement, the patients with acromegaly had significantly lower levels of osteocalcin ($P=0.003$) and β-crosslaps ($P=0.007$) than before the treatment. Activity of bone alkaline phosphatase tended to lower, but the difference was not significant between the two measurements. No differences were found in osteocalcin level between the patients in remission and the control group ($P=0.09$), while the crosslaps level as well as the activity of alkaline phosphatase was still significantly higher in the acromegaly remission group ($P=0.04$ for both parameters).
Conclusion
In patients with acromegaly, 6 months after achievement of disease remission, bone turnover significantly decreases, but still remains higher than in healthy individuals.

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P916

Skin flora in acromegaly

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Introduction
Recent study showed that patients with acromegaly have typical skin findings including increased sebum secretion, decreased transepidermal water loss, more alkaline, and colder skin surface correlated with serum GH and IGF1 levels.
The aim of this study was to demonstrate the effect of functional skin changes on the skin flora in patients with acromegaly.
Methods and designs
This case–control study was conducted in university hospitals in Mersin, Turkey, study consisted of 30 acromegalic patients and 60 healty adults who had no previously diagnosed chronic illness as a control group.
A total of 90 volunteers were enrolled in a cohort; nasal and axillar cultures were obtanied. Axillar and nasal specimens from anterior nares of the individuals were taken using sterile swabs.
Results
Nasal colonization of *Staphylococcus aureus* was 13.3% in acromegalic patients, but 43.4% in control group this differences was statistically significant ($P=0.004$). Patients and control group compared according to axillar and nasal cultures, we determined proteus colonisation 16.7% in patients with acromegaly but no proteus colonisation in control group. This result was statistically significant ($P=0.001$). Proteus colonization was negatively corellated only with disease duration in acromegalic patients ($P=0.017$).
Conclusion
Normally, very little water is present on the skin surface and skin forms an acid mantle. But acromegaly associated with increased sebum secretion, decreased transepidermal water loss, more alkaline, and colder skin surface. Skin colonization by proteus may be due to increased skin alkaline mantle in acromegaly.

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P917

Effect of short- and long-term treatment with pasireotide on hemochrome in patients with Cushing's disease

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Introduction
Glucocorticoids (GC) have a stimulatory effect on neutrophils and an inhibitory effect on the other leukocyte subpopulations. A potential stimulatory effect on erythropoiesis has been also hypothesized. The aim of our study was to evaluate the effect of pasireotide treatment on hemochrome parameters in patients with endogenous pituitary-dependent glucocorticoid excess or Cushing's disease (CD).
Patients and methods
Fifteen patients with CD (19–57 years, 14 F, 1 M) and 45 sex-, age- and BMI-matched healthy controls entered the study. Hemochrome evaluation has been assessed at baseline and after 3 and 6 months of pasireotide treatment (dose 1200–2400 μg/day).
Results
Significantly higher levels of hematocrit (HCT) ($P=0.019$) and neutrophils ($P=0.002$) and a significantly lower number of lymphocytes ($P=0.000$) and eosinophils ($P=0.009$) have been observed in patients than controls. After 3 months of treatment, 8/15 patients (53.3%) normalized urinary free cortisol

(UFC) levels and the percentage of reduction in UFC levels ranged from 25 to 96%; a significant decrease in platelets (PLT) ($P=0.002$) and neutrophils ($P=0.017$) and also a significant increase in lymphocytes ($P=0.03$), eosinophils ($P=0.035$) and basophils ($P=0.011$) have been observed. After 6 months, 5/13 patients (38.5%) had a persistent UFC normalization and the percentage of reduction in UFC levels ranged from 30 to 91%; so as after 3 months, a significant decrease in PLT ($P=0.015$) and neutrophils ($P=0.023$) and a significant increase in lymphocytes ($P=0.002$) and basophils ($P=0.034$) have been observed. No significant correlation was found between UFC levels and the different parameters of hemochrome at baseline and no significant correlation was found between their changes after 3 and 6 months of treatment.
Conclusions
Medical therapy with pasireotide is able to improve hemochrome parameters commonly altered in CD. These findings are probably due to a significant decrease in cortisol secretion, although a contributing or determinant role of different factors cannot be ruled out.

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Evaluation of QT dispersion at the time of diagnosis and at the end of follow-up in acromegaly patients

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Background
In this study we have aimed to calculate the QT dispersion in acromegaly patients and reveal its correlation with GH and IGF1.
Materials and methods
Forty-one acromegaly patients were enrolled in the study. Another 41 patients with similar age, sex and comorbid disease distrubition have constituted the control group. We have evaluated the electrocardiograms (ECG) of the acromegaly patients at the time of diagnosis (baseline) and at the end of follow-up (post follow-up). Only one ECG was provided from each patient in the control group. The longest (QT max), the shortest QT (QT min), QT dispersion, corrected QT max (QTc max), QTc min and QTc dispersion were calculated.
Results
Baseline QT max, QT dispersion, QTc max and QTc dispersion intervals were significantly longer than the control group (respectively; $P=0.016$, $P=0.001$, $P=0.001$, $P=0.002$). QTc max and QTc dispersion intervals were significantly shorter at the end of the follow-up period compared to baseline, in acromegaly patients (respectively; $P=0.005$, $P=0.024$). Post follow-up QT intervals were not statistically different from the control group. Except the negative correlation between GH and QTc dispersion of acromegaly patients in post follow-up period, we could not detect any other correlation between QT intervals and GH or IGF1 levels. QTc dispersion was found to be related with disease duration in acromegaly group ($r=0.440$, $P=0.009$).
Conclusions
According to the findings of this study we can claim that QT intervals are beneficial in determining the arrhythmia risk in acromegaly patients and this risk can be reduced after treatment and hormonal control.

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Transsphenoidal surgery, gamma-knife surgery and diabetes are the factors affecting the quality of life in acromegalic patients

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Acromegaly is a chronic disease with an important impact on patients, health-related quality of life (HRQoL). Acromegaly quality of life questionnaire (AcroQoL) is a disease-generated QoL questionnaire comprising 22 questions each having five possible responses scored 1–5, the maximum score of 110 reflecting best possible QOL, and quoted as a percentage. The 22 items break down into two categories, physical and psychological function, the latter being subdivided into appearance and personal relationships. We aimed to investigate the factors affecting the QoL in acromegalic patients by using AcroQoL. We have performed a cross-sectional study in 65 acromegalic patients (37 males, 28 females). Age (mean$\pm$S.D.: 44.75$\pm$10.81 years), BMI (mean$\pm$S.D.: 29.77 $\pm$4.20 kg/m^2), disease duration (median (IQR): 48 (12–103) months), basal GH (median (IQR): 1.66 (0.69–6.05) ng/dl), nadir GH (median (IQR): 1.13 (0.47–4.80) ng/dl), and IGF1 (median (IQR): 311 (199–504.5) ng/dl) were evaluated. Comorbidities were as follows: diabetes 27.7%, hypertension 43.1%, hyperlipidemia 32.3%, and hypopituitarism 29.2%. 55 patients (84.6%) underwent transsphenoidal surgery (TSS), gamma-knife radiosurgery (GKS) was performed in 39 (60%) patients and 41 (63.1%) patients were on somatostatin anlogue (SA) treatment. Disease control was evaluated by IGF1 (referance values per age) and GH levels (random GH <1 ng/dl in patients on SA and nadir GH <0.4 ng/dl in patients not on SA). 24 (36.9%) patients were under control according to IGF1 criteria, while 20 (30.8%) patients. were under control according to GH criteria. No correlation was found between AcroQoL scores and any of the parameters. AcroQoL scores were lower in patients with diabetes (52 vs 67, $P<0.05$) and who had radiotherapy (59 vs 75, $P<0.05$). However, patients who underwent TSS had higher scores (66 vs 39, $P<0.05$). In conclusion, TSS, GKS and co-existing diabetes were the only factors affecting acromegaly related QoL.

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The short synacthen test may be more sensitive than the glucagon stimulation test in assessing the hypothalamic–pituitary–adrenal axis: a retrospective audit

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Introduction
Insulin tolerance test (ITT) is considered as the gold standard assessment for hypothalamic–pituitary–adrenal axis (HPA) integrity. Short synacthen test (SST) is a relatively simple, low-cost and well tolerated first line test of HPA despite concerns regarding accuracy. The glucagon stimulation test (GST) is often used as an alternative to the ITT. Although less reliable, it is particularly useful when insulin-induced hypoglycaemia is contra-indicated. This retrospective audit set to evaluate accuracy of the SST and GST against the ITT with the aim of advising on the best method of HPA investigation.
Method
Patients who underwent ITT between 2009 and 2012 and had at least one screening test were audited. The ITT was considered as gold standard and pre-screening SST and/or GST results were compared against it.
Results
patients underwent ITT of whom 31 had pre-screening SST and/or GST. 21 were females and average age was 42.9. 23 with clinically suspected hypopituitarism; six post-transphenoidal surgery and two with none ACTH-producing pituitary adenoma. 26 of the 31 patients underwent both SST and ITT. 13 patients failed both SST and ITT and only one patient passed SST but failed ITT giving an SST sensitivity of 92.8% (95% CI 86.1–99%) and a specificity of 8.3% (95% CI 4–16.2%). Six out of 16 patients underwent both GST and ITT failed both tests, while two passed GST but failed ITT giving GST sensitivity of 75% (95% CI 75–94.7%) and a specificity of 0% (95% CI 0–19.7%). Measure of agreement between SST and GST was poor ($\kappa=-0.13$).
Conclusion
SST is more sensitive and specific than GST for assessing HPA when compared to the gold standard ITT.
Recommendation
SST is a more appropriate screening test for HPA axis and should replace the GST when ITT is contraindicated.

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Abstract withdrawn.

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Characterization of GH-producing pituitary adenomas according to responsiveness to thyrotropin-releasing hormone

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Objective
The relationship between the paradoxical response of GH secretion after thyrotropin-releasing hormone (TRH) administration and tumor size has been the subject of only a few studies with conflicting results.
Design
The aim of this study was to investigate how the paradoxical response of GH secretion to TRH changes according to tumor size.
Methods
Patients with newly diagnosed acromegaly were classified as either TRH responders or non-responders according to the results of a TRH stimulation test (TST). GH levels of patients during the TST were compared according to responsiveness to TRH and tumor size. The relationship between $\Delta GH_{max-min}$ (the difference between peak and basal GH levels during the TST) and tumor size was investigated. Lastly, tumor volumes were compared between TRH responders and non-responders.
Results
A total of 112 acromegalic patients who underwent the TST were included in this study. TRH responders showed significantly higher GH levels than non-responders during the entire TST time. $\Delta GH_{max-min}$ during the TST was higher in TRH responders than non-responders, and tumor volumes were likely to be greater in responders than non-responders, although this difference was not statistically significant. Among 69 patients who remained after excluding patients without results of other tests or sellar MR images, those with macroadenomas demonstrated higher GH levels during the entire TST time. Both peak GH levels and $\Delta GH_{max-min}$ during the TST showed significant correlations with tumor volume.
Conclusion
The paradoxical response of GH secretion to TRH in GH-producing pituitary adenomas was positively correlated with tumor size.

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Tumour volume reduction and GH/IGF1 control with lanreotide Autogel 120 mg every 28 days for treatment-naïve acromegalic patients with GH-secreting pituitary macroadenoma: the PRIMARYS study

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Introduction
Surgery is recognized first-line therapy for acromegalic patients but tumour size and GH/IGF1 control has also been observed after primary somatostatin analogue treatment. This is the first study of therapy with high-dose lanreotide Autogel over one year in a large cohort of treatment-naïve acromegalic patients.
Methods
In this international, multicentre, open-label, single-arm, phase 3b study (NCT00690898/EudraCT2007-000155-34), treatment-naïve acromegalic patients with GH-secreting pituitary macroadenoma received primary therapy with lanreotide Autogel 120 mg every 28 days for 48 weeks. The primary endpoint was percentage of patients with $\geq$20% tumour volume reduction (baseline, week 48) based on MRI central assessments from three readers. The primary analysis used the reader with best standardized sensitivity determined on repeatability tests.
Results
Ninety patients received treatment (baseline mean maximum adenoma diameter 19.0 mm (range 10.6–50.4 mm), tumour volume 2739 mm^3, GH 15.0 μg/l, IGF1 810 μg/l). In the intention-to-treat population, the primary analysis showed 56/89 (63%) patients achieved $\geq$20% tumour volume reduction (95% CI: 52–73%). In the per-protocol population, the same reader found $\geq$20% tumour reduction in 47/63 patients (75% (62–85%)). Based on last available assessments, GH levels $\leq$2.5 μg/l were achieved in 58/89 (65% (54–75%)), IGF1 within age/sex-matched normal range in 34/88 (39% (28–50%)), and GH/IGF1 control in 30/88 (34% (24–45%)). Lanreotide was well-tolerated, most patients reported mild AEs (56/90 (62%)) and/or moderate AEs (36/90 (40%)), and only 5/90 discontinued due to AEs (6%). Gastrointestinal disorders were most frequent AEs (58/90 (64%)).
Conclusions
In patients with newly-diagnosed GH-secreting pituitary macroadenoma, primary therapy with lanreotide Autogel at 120 mg every 28 days achieved clinically-relevant reduction in pituitary adenoma volume and sustained GH/IGF1 control with a favourable safety profile over 48 weeks' follow-up. These data support further exploring the potential use of lanreotide as an initial therapeutic option in this patient population.

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Endoscopic transsphenoidal surgery for acromegaly: assessing the surgical outcome based on current criteria of remission

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Introduction
Acromegaly is associated with increased morbidity and mortality, mainly due to cardiovascular and metabolic complications and higher risk of malignancy. The treatment of choice is selective transsphenoidal surgery (TSS). Successful removal of somatotroph adenoma normalizes GH and IGF1 levels, which improves the patients prognosis. The efficacy of TSS depends on the tumor volume, parasellar extension and surgeon experience.
The aim of this study was to assess the safety and efficacy of pure, endoscopic TSS based on the stringent criteria of remission.
Material and methods
Retrospective study involving 25 consecutive patients with GH-secreting pituitary adenoma (9 males and 16 females). The mean age was 45.04 years. All subjects underwent pure endoscopic TSS in 2010. They were all operated on by the same neurosurgeon and according to the same surgical protocol. Diagnosis of acromegaly was based on commonly adopted hormonal criteria and magnetic resonance imaging (MRI). Lateral invasion to the cavernous sinuses was classified according to Knosp's scale. Remission was defined as nadir GH $\leq$0.4 ng/ml following 75.0 of oral glucose and IGF1 within the referral limits for age and gender or random GH $\leq$1 ng/ml.
Results
The MRI precisely visualized 18 macroadenomas (72%) and 7 microadenomas (28%) in the study group. Remission was achieved in 11 subjects with macroadenomas (61.1%) and in six patients with microadenomas (85.7%). Intraoperative, cerebrospinal fluid leakage was observed in six subjects with pituitary macroadenoma. Additionally, one case of epistaxis and one sinusitis was found. There were neither meningitis nor persistent CSF leakage. The permanent diabetes insipidus and anterior pituitary insufficiency was observed in case of one patient.
Conclusions
Pure endoscopic TSS was an effective and safe procedure. The main limitation of complete tumor removal was cavernous sinuses involvement.

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P925

The influence of octreotide-LAR treatment on glucose homeostasis in acromegaly

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Introduction
Impaired glucose tolerance and insulin resistance are frequently associated with acromegaly. The aim of this study was to assess the effect of octreotide-LAR treatment on glucose homeostasis in acromegalic patients.
Patients and methods
In this prospective study 16 naïve acromegalic patients were studied before and after 3-month therapy of octreotide-LAR (20 mg i.m. every 28 days). Diagnosis of active acromegaly was established on the basis of widely recognized criteria. In each patient glucose and insulin concentrations were assessed during the 75 g oral glucose tolerance test (OGTT) and HbA1c levels were measured. To estimate insulin sensitivity hyperinsulinemic euglycemic clamp method was used and homeostasis model assessment (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.

Results
After 3 months of treatment no statistically significant change in plasma glucose levels both fasting and during OGTT ($P>0.05$) was found, but a significant reduction in HbA1c level ($6.54\pm1.72\%$ vs $6.02\pm0.78\%$) was noticed. A prominent reduction in insulin secretion was found after octreotide-LAR treatment compared to the moment of diagnosis (4.4 ± 2.0 vs 12.1 ± 9.6 mIU/ml, $P<0.001$). After treatment, there was a significant reduction in HOMA-IR (0.92 vs 2.27, $P<0.05$) and a significant increase in QUICKI (0.39 vs 0.34, $P<0.05$). In euglycemic clamp method a statistically significant increase in M value (4.52 ± 2.34 vs 2.37 ± 1.24 mg/kg per min) was noticed.
Conclusions
We concluded that in acromegalic patients octreotide-LAR therapy significantly improves glucose homeostasis by reducing insulin resistance.

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Somatotropin and IGF1 levels at diagnose and after surgery in acromegalic patients: is it possible to predict the likelihood of cure at diagnosis?

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Introduction
Elevated levels of somatotropin (ST) and IGF1 in acromegalic patients are associated with high morbidity and mortality. The normalization of ST and IGF1 allows the reversal of most of their negative effects.
Objective
To evaluate characteristics present at diagnosis, predictive of cure, after surgery, in patients with ST-producing tumors.
Methods
Retrospective study including acromegalic patients diagnosed between 1982 and 2012. Patients that underwent surgery and postoperative evaluation were selected. Data regarding tumor characteristics, ST nadir, IGF1 levels and clinical parameters was collected. Independent samples t-test and partial correlation were used for statistical analysis.
Results
We evaluated 47 patients with ST-producing tumors. Mean age at diagnose was 42.0 ± 12.4 years, delay in diagnosis 6.0 ± 4.4 years, tumor size (considering the largest diameter) 18.7 ± 12.6 mm, ST nadir, prior to surgery, 19.2 ± 24.3 ng/ml, and after surgery 5.1 ± 12.0 ng/ml. Patients with cure criteria after surgery were compared with patients with persistent disease (ST nadir after surgery 0.39 ± 0.36 vs 10.24 ± 16.78 ng/ml, $P=0.025$). No differences were found between the two groups relating the delay in diagnosis (6.1 ± 5.1 vs 6.1 ± 4.4 years, $P=0.985$), prolactin levels (44.2 ± 98.1 vs 45.9 ± 52.3 ng/ml, $P=0.946$), IGF1 (807.3 ± 344.9 vs 744.4 ± 323.4 ng/ml, $P=0.576$) and tumor size (18.6 ± 14.1 vs 18.7 ± 8.7 mm, $P=0.982$). Differences were found regarding age at diagnosis (48.2 ± 14.3 vs 40.0 ± 9.3 years, $P=0.04$) and ST nadir before surgery (10.8 ± 9.4 vs 21.9 ± 19.2, $P=0.047$), this with moderate correlation with postoperative nadir ($r=0.415$, $P=0.008$), after controlling for age. There were no differences in the frequency of symptoms and manifestations of the disease between the two groups.
Discussion
Higher levels of ST nadir at diagnostic OGTT confer greater risk of persistent disease after surgery.

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Therapeutic response to recombinat somatropin in children with isolated deficiency of GH

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Introduction
Treatment with recombinant ST is subject to strict criteria. Differences in the response to therapy based on the maximum level of ST in diagnostic tests (ST ≤5 vs ST >5 ng/ml), could predict whose patients would benefit more from therapy.
Purpase
Assess differences in the response to therapy with rST in patients with IDGH.
Methods
Selected 18 patients with IDGH followed by Endocrinology. Anthropometric (height (H), weight, growth velocity (GV), target height (TH)), analytical (IGF1, IGFBP3) and imaging (bone age (BA)) data were collected before, and 12 months after treatment. Two groups were formed based in ST levels obtained in the stimulation tests, ST ≤5 ng/ml ($n=9$) and ST >5 ng/ml.
Results
The sample (male $n=13$) presented chronological age (CA) of 8.6 ± 3.5 years, H 114.3 ± 18.6 cm (z-score -2.7 ± 0.6), BMI 17.3 ± 3.1 kg/m^2; TH 165.4 ± 7.3 cm; BA 5.8 ± 3.5 years; GV 4.0 ± 0.8 cm/year; IGF1 104.7 ± 70.1 ng/ml, IGFBP3 2.8 ± 1.3 mg/ml. After 12 months of therapy there was a significant increase in GV (8.1 ± 2.0, $P<0.001$) and H (123.9 ± 19.2, $P<0.001$; z-score -2.0 ± 0.9). When comparing both groups, there was a difference in ST levels after stimulation tests (3.4 ± 1.8 vs 5.9 ± 1.1 ng/ml, $P=0.03$). There were no other differences between the two groups when comparing for dose of rST (0.035 ± 0.008 vs 0.035 ± 0.005 mg/kg per day, $P=0.867$), anthropometric (H 114.4 ± 21.2 vs 114.1 ± 16.9 cm, $P=0.98$; GV 3.8 ± 0.7 vs 4.2 ± 0.9 cm/year, $P=0.28$), analytical (IGF1 104.1 ± 77.1 vs 105.3 ± 68.1 ng/ml, $P=0.97$) or imaging (BA 5.9 ± 3.9 vs 5.7 ± 3.2 years; $P=0.90$) parameters before treatment. The same was observed 12 months after therapy (ST dose 0.031 ± 0.008 vs 0.029 ± 0.004 mg/kg per day, $P=0.42$; H 124.4 ± 22.2 vs 123.5 ± 16.6 cm, $P=0.93$; GV 8.5 ± 2.5 vs 7.7 ± 1.4 cm/year, $P=0.44$; IGF1 258.8 ± 243.4 vs 309.6 ± 191.4 ng/ml, $P=0.63$).
Conclusion
Response to therapy with rST in children with IDGH is similar in cases of severe and in cases of partial deficit.

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Thyroid cancer in patients with acromegaly

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It is known that acromegaly is associated with increased risk of benign and malignant tumours. Previous studies show the prevalence of nodular thyroid disease in 40–70% of patients with acromegaly, and a 5–7% prevalence of thyroid cancer in these patients, which is higher compared to the general population.
Latvian Cancer Register data show an incidence of thyroid cancer of 7.5–7.7 per 100 000 inhabitants in 2007–2010.
We retrospectively studied our hospital acromegaly database of 60 patients. Only 10 patients (16%) had nodular goiter, 9 of them underwent thyroid surgery, and in 4 cases (two females and two males) histological examination revealed thyroid cancer (papillary carcinoma in all). It means 6.6% of acromegaly patients had thyroid cancer. Three of four patients with thyroid malignancy, and one of five patients with benign nodules had an active acromegaly at the time of thyroid surgery.
Genetic testing of four acromegaly patients with papillary thyroid cancer revealed the presence of *SSTR5* gene polymorphism rs34037914 T alleles (genotypes TT and TG) in two patients, which is associated with increased risk of acromegaly. Our results point to a certain association of thyroid cancer with acromegaly activity. The post-operative observation period is too short to allow evaluation of the rate of possible thyroid cancer relapse, especially in patients with consistenly increased GH and IGF1. Patients with acromegaly and nodular goiter should be often controled by thyroid ultrasound followed by fine needle aspiration biopsy as indicated.

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Therapeutic role of dopamine-agonists in ESRF-induced hyperprolactinaemia

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Moderate hyperprolactinaemia (<1000 mU/l) is a common abnormal biochemical finding in patients with end-stage renal failure (ESRF). The underlying

pathophysiological mechanism is thought to be due to a combination of increased prolactin secretion as well as delayed renal clearance. There are no current clear guidelines for the treatment of ESRF-induced symptomatic hyperprolactinaemia but renal transplantation has been shown to reverse the raised prolactin back to normal. We describe two cases whereby dopamine-agonist treatment induced regression of prolactin-related signs and symptoms in ESRF.

A 69-year-old man with a history of ESRF due to focal segmental glomerulosclerosis on haemodialysis presented with bilateral gynaecomastia to the endocrine clinic. Investigations revealed a grossly elevated serum prolactin of 19 573 mU/l and a low serum testosterone level of 3.0 nmol/l. CT scan of pituitary (patient had permanent pacemaker *in situ*) showed a bulky pituitary fossa but no obvious macroadenoma. He was commenced on cabergoline following which his prolactin promptly normalised with subsequent resolution of his breast symptoms. CT pituitary findings remained unchanged however.

A 53-year-old man on haemodialysis for ESRF secondary to resistant hypertension complained of reduced libido, erectile dysfunction and painful bilateral gynaecomastia. Investigations showed a low testosterone level of 6.3 nmol/l with an elevated prolactin levels of 2683 mU/l which was initially thought to be secondary to ESRF. MRI of his pituitary showed a thickened pituitary stalk. He was commenced on Bromocriptine which normalised his prolactin and he improved symptomatically. His serum testosterone however remained low.

These cases illustrate that dopamine agonists clearly have a therapeutic benefit in ESRF-induced hyperprolactinaemic breast symptoms and should be considered as a potential useful medical therapy target. They do not however seem to improve hypogonadism. This would suggest a different mechanism affecting disturbance of the neuroendocrine hypogonad system by ESRF.

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P930

A rare case of pituitary infarction in an 11-year-old pre-pubertal girl with pituitary autoantibodies to piccolo

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Introduction

Pituitary infarction is rare in the paediatric age. In adolescents and adults it is often secondary to haemorrhage into an underlying lesion such as pituitary adenoma or cyst. Lymphocytic hypophysitis is rare in children and usually peri-tumoral due to germinoma, craniopharyngioma or Rathke's cyst and often associated with hypopituitarism and diabetes insipidus.

Case report

An 11-year-old girl was admitted with a urinary tract infection (UTI) and sudden onset vomiting. She also had experienced frontal headaches, fever, low blood pressure, lethargy and 6 kg weight loss of 3 weeks duration. Symptoms seemed disproportionate to a UTI alone. Laboratory tests indicated normal electrolytes but low TSH 0.09 mIU/l (*n* 0.6–3.7) and morning cortisol <30 nmol/l ($n>250$) and mild elevation Prolactin 60 (*n* 0–42). MRI showed a normal sized anterior pituitary 7 mm with loss of the pituitary bright spot. After gadolinium, normal stalk but no enhancement on the delayed images suggesting a cystic lesion or infarction of the pituitary. MRI 1 month later showed the pituitary had halved in size and over 2 years progressed to an 'empty sella'. Tumour markers, viral serology and tuberculosis screen were negative. Adrenal antibodies were negative. Pituitary autoantibodies by ITT (*in-vitro* transcription translation) assay for TPIT, CADPS, CHD8 and enolase were negative but positive for piccolo, a protein involved in dense-core vesicle transport. Hydrocortisone, thyroxine and GH replacement therapy was started and subsequently pubertal induction needed. She remains hypopituitary 10 years later.

Conclusions

No definite cause for pituitary infarction was found in an 11-year-old girl but pituitary autoantibodies reacted to the protein piccolo. We suspect this patient had hypophysitis related to a cystic lesion with resulting infarction of the pituitary. Pituitary autoantibodies may be epiphenomena and not causative.

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Idiopathic isolated ACTH deficiency presenting with severe hyponatraemia

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Introduction

Acquired isolated ACTH deficiency with normal secretion of the remaining pituitary hormones is a rare condition in the absence of exposure to exogenous glucocorticoids. The aetiology is assumed to be autoimmune in most adult cases with traumatic brain injury and radiation exposure being rare causes. Pituitary transcription factor mutations may be identified in congenital and childhood onset cases. Hyponatraemia is typically associated with primary adrenal insufficiency due to a lack of aldosterone, but is rarely encountered in secondary adrenal insufficiency.

Case report

We report three patients (two females and one male, age 58–82 years) presenting with moderate to severe hyponatraemia (114–127 mmol/l) as the initial and principal manifestation leading to the diagnosis of secondary adrenal insufficiency due to isolated ACTH deficiency (serum cortisol 20–113 nmol/l, ACTH 5–20 ng/l). All patients reported longstanding (1–10 years) fatigue and weakness retrospectively. Relapsing hyponatraemia had been previously documented in one patient. The further workup did not reveal deficiencies of other pituitary hormones and sellar MRI scans were normal in two patients and showed a partially empty sella in the remaining. Hydrocortisone replacement resulted in rapid resolution of hyponatraemia and clinical improvement in every patient.

Conclusion

Secondary adrenal insufficiency due to isolated ACTH deficiency, although rare, must be included in the differential diagnosis of hyponatraemia. Enhanced vasopressin release due to a lack of cortisol mediated negative feedback suppression is the likely pathogenetic mechanism leading to euvolemic hyponatraemia. Associated autoimmune diseases may point to the underlying mechanism of corticotroph dysfunction but the etiology may remain obscure despite extensive workup. Hydrocortisone replacement results in rapid clinical and biochemical improvement and should promptly be installed.

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P932

Diagnostic features and surgical therapy of acromegalic patients: experience of the last three decades

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Introduction

Acromegaly is a rare and insidious disease associated with an increased morbidity and mortality. Trans-sphenoidal (TNS) surgery remains the primary therapeutic option, in particular for intrasellar microadenomas and noninvasive macro-adenomas. Aims of this study were to describe diagnostic features and to verify the impact of TNS surgery on treatment of acromegaly over three decades, before and after the identification of a dedicated neurosurgical team.

Design and methods

Forty-nine patients (group A) who underwent TNS surgery by a dedicated neurosurgical team between 2000 and 2010, and 126 patients (group B) who underwent TNS approach by a not selected operator between 1979 and 1999 were retrospectively analyzed.

Results

At baseline, 67% of patients of group A and 69% of patients of group B were macroadenomas. The mean delay of diagnosis was 5.5 and 5.9 years in group A

and B, respectively. Moreover, no significant differences between two groups in terms of mean basal GH levels, mean GH nadir values, prevalence of hypopituitarism and hypertension were observed. IGF1 SDS were significantly higher, while BMI and prevalence of IGT/diabetes were significantly lower in group B than in group A. After surgery, overall remission rate was 53% in patients of group A (75% in microadenomas and 42% in macroadenomas, $P<0.05$) and 37% in patients of group B ($P=0.08$ vs group A; for microadenomas, 34 vs 75% of group A, $P<0.05$, for macroadenomas, 36 vs 42% of group A, $P=$NS).
Conclusions
Our data confirm that a dedicated neurosurgical team is needed in order to improve remission rates of surgery in acromegalic patients. However, we do not observe significant changes in biochemical, clinical and neuroradiological presentation of disease over the last three decades. As the high prevalence of macroadenomas negatively influence surgical cure, earlier diagnosis should be considered mandatory to achieve a better outcome.

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P933

Pituitary tumours and epilepsy

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Introduction
Epilepsy is a heterogeneous condition with different aetiologies including genetics, cerebral trauma, toxic exposures, infection, vascular diseases and neoplasms. Among the last ones, pituitary tumours (PT) are rarely responsible of convulsions, except when they are very large invading the nervous system. Our aim is to analyze the conditions under which the epilepsy appears and disappears in people with pituitary tumours.
Subjects and methods
We have analyzed eight subjects who were hospitalized in our department for pituitary tumours and epilepsy. After questioning them, they were examined, and then had biological, hormonal and radiological assessment based on MRI.
Among this group we had six pure PRL, one adenoma secreting prolactin and GH and the 8th one was a non functioning pituitary tumour. Chronic epileptic crisis was the consultation motive in 7/8 cases although there were other symptoms such as visual and sexual troubles. In one case acute epileptic crisis appears during radiotherapy. All were males, mean age=33.75 years (22–58), mean prolactin=9198 ng/ml and tumour's height was >40 mm in all except one. The temporal lobe was invaded in six cases. After tumour volume reduction (by surgery $n=1$, medications $n=7$), epilepsy disappeared and epileptic drugs were stopped except in one recent case.
Conclusion
Epilepsy which is a life threatening condition can reveal some pituitary tumours, especially large somatolactotrop or pure prolactinomas. It can also appear under aggressive treatment such as radiotherapy. That one should be avoided before tumour reduction by surgery or medical treatment if possible.

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P934

Predictors of hormonal status after pituitary surgery

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Introduction
Surgical treatment of pituitary adenomas (PA) may affect hormone situation.
Objective
To evaluate the frequency of hypopituitarism and investigate the potential predictors of worsening or improving pituitary function after PA surgery.
Methods
Retrospective analysis of all PA operated in our hospital between 2001 and 2010.
Results
Eighty patients were included (51.2% women), with a mean age of 55.6 years (s.d. 16.6 years); 71.2% had non functioning PA. 95% were macroadenomas and mean tumor size was 25.8 mm (s.d. 14 mm). Preoperative hormonal status was normal in 45.3%, 18.8% had 1–2 pituitary deficits and 35.9% had ≥ 3 deficits. After the intervention, pituitary function was improved or no changed in 70.4% of patients (41.8% had a normal function, 20% 1–2 deficits and 38.2% ≥ 3 deficits). Predictors of hormone impairment: on univariate analysis we found a non significant trend to worsening in macroadenomas, PA with extrasellar extension and patients with postoperative cerebrospinal fluid fistula. In the last 5 years, the percentage of hormone impairment was reduced (44.4 vs 19.2%, $P=0.07$). Tumor size and invasion were significant and independently associated to TSH, GH and FSH/LH postsurgical deficits. A normal basal pituitary function was the main conditioning of a normal postsurgical pituitary status. The 85.7% of patients with normal postoperative function had a normal preoperative function too ($P<0.001$).
On multivariate analysis with logistic regression, size, PA functionality and the number of presurgical deficits were predictors of hormone improving. On the other hand, surgery in the first 5 years of the study period was related to hormone impairment.
Conclusions
Tumor size and invasion were the most important predictors of postsurgical hypopituitarism in our series. Smaller lessions, with intrasellar location or with basal pituitary normofunction exhibited the best outcomes. The greatest experience of our neurosurgical team could explain the better results obtained in the last years.

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P935

Fetuin-A can be used as a marker of acromegaly

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Introduction
Acromegaly is a rare, chronic disorder, with classical clinical features caused by excess GH and consequent raised levels IGF1 and is associated with increased morbidity and mortality. Early diagnosis of the disease is very important for patient survival. Fetuin-A is a plasma glycoprotein that is produced by hepatocytes and it is the most important systemic calcification inhibitor. The physiological importance of fetuin-A, in acromegaly remains obscure. The aim of this study was to investigate the effect of fetuin-A levels on atherosclerotic process in acromegalic patients.
Methods
In this study 37 acromegalic patients and 30 controls were included to the study. Antropometric, biochemical and hematological findings were examined. Height, weight, BMI, and blood pressure of the groups were noted. Fasting blood glucose, lipid profile, GH, IGF1, IGFBP3, TSH, insulin levels were measured. Fetuin-A levels were measured by Elisa method.
Results
We observed significantly higher Fetuin-A levels in acromegalic patients ($P<0.001$). There was no correlation between fetuin-A and GH, IGFBP3, HOMA, BMI, TG, T. kol, HDL and LDL levels. It was found positive correlation between Fetuin-A andIGF1 levels ($P<0.05$).
Conclusion
We observed significantly higher fetuin-A levels in acromegalic patients. Our study was the first study that evaluates fetuin-A levels in acromegalic patients. We found positive correlation with fetuin-A and IGF1, in acromegalic patients.

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P936

Evaluation of body weight, insulin resistance, leptin and adiponectin levels in premenopausal women with hyperprolactinemia

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Introduction
The effects of hyperprolactinemia on metabolic parameters are not conclusive and a few data evaluating adiponectin levels in prolactinoma and idiopathic hyperprolactinemia exist. The aim of this study is to evaluate effects of hyperprolactinemia on body weight, insulin resistance, β cell function, leptin and adiponectin levels in premenopausal women with hyperprolactinemia.
Methods and design
Forty premenopausal women with prolactinoma or idiopathic hyperprolactinemia were compared to 41 age-matched healthy premenopausal women with regard to body weight, BMI, waist and hip circumferences, waist to hip ratio, fasting plasma glucose, insulin, insulin resistance measured by homeostasis model assessment-insulin resistance index (HOMA-IR), β cell function measured by homeostasis model assessment-β index (HOMA-β), leptin and adiponectin levels.
Results
Plasma insulin levels, HOMA-IR and HOMA-β were significantly higher in hyperprolactinemic women than the control group ($P=0.012$, 0.01 and 0.005, respectively). The other parameters were not significantly different from the control group. There was a positive correlation between prolactin levels and fasting plasma glucose ($P=0.025$, $r=0.354$).
Conclusions
The results of the study showed that high prolactin levels may be associated with hyperinsulinemia and insulin resistance in premenopausal women. This effect seems to be independent from body weight, leptin and adiponectin levels. High prolactin levels may directly stimulate insulin secretion from pancreas and directly cause hepatic and whole-body insulin resistance. Correlation between prolactin and fasting plasma glucose suggests an increased hepatic glucose output by prolactin and thus may indicate an increased hepatic insulin resistance.

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P937

Carbohydrate metabolism in acromegaly and treatment impact

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Introduction
Carbohydrate metabolism (CHM) is impaired in over 30% of acromegalic patients. Natural history of acromegaly and treatment modalities, i.e. surgery, somatostatin analogues (SSA) and pegvisomant, may impact in a different way on CHM.
Aim
To assess CHM alterations (impaired fasting glucose (IFG) and diabetes mellitus (DM)) in acromegaly and their relationship with clinical features and treatment options.
Patients and methods
In a retrospective study we have included 55 patients, with acromegaly. Age, gender, BMI, tumor size, IGF1 levels and the presence of IFG or DM have been analyzed before and after surgery or medical treatment.
Results
There were 30 men and 25 women. Mean age before treatment was 57 ± 17 years and mean BMI was 28 ± 3.8 kg/m^2. We have found IFG in 13 and DM in 15 patients (total 28; 50%). We have found no statistically significant differences in age, gender, BMI and IGF1 levels between IFG/DM and patients without CHM impairment. However, IFG/DM patients have more frequently macroadenomas. Transsphenoidal resection was performed in 49 (88%) cases. Because of persistence of high postsurgical IGF1 levels ($n=24$) or as a primary therapy ($n=6$), 30 patients received SSA. For persistence of high IGF1 levels, nine cases were shifted to pegvisomant.
In diabetic patients, HbA1c decreased after surgery from 7.6% (6.7–8.5) to 6.7% (6.2–6.8) and after SSA from 7.1% (6.7–7.4) to 6.6% (5.7–8.5), but only in patients on pegvisomant we have observed a significant reduction HbA1c from 9.8% (8.9–10.5) to 5.6% (5.5–6.8). Furthermore, only in pegvisomant group, we had to lower insulin and/or oral agents whereas with surgery or SSA the diabetic treatment requirement was higher.
Conclusions
Up to 50% of patients with active acromegaly have CHM impairment and correlates with tumor size. Only pegvisomant is associated with significant improvement in glycemic control and a reduction in hypoglycemic treatment.

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P938

Pituitary apoplexy in clinically nonfunctioning pituitary adenomas: experience of a single center

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Introduction
Clinically nonfunctioning pituitary adenomas (CNFPA) represent 25–50% of all pituitary lesions. They may be asymptomatic and discovered incidentally or they may be diagnosed due to visual impairment or symptoms of pituitary hormone insufficiency. There is little data on apoplexy frequency in the CNFPA.
The aim of our study was to assess the frequency of CNFPA among all pituitary adenomas and additionally to evaluate how common is apoplexy as an initial presentation in these adenomas.
Design
We studied retrospectively all patients who were admitted and diagnosed with a pituitary adenoma in our department, during the last 10 years. For this purpose, patients' charts were reviewed for clinical features upon admission, laboratory and magnetic resonance findings during hospitalization and histopathological results after surgical treatment.
Results
We identified 116 patients with pituitary adenomas and 42 (27.6%) of them had a CNFPA (26 men and 16 women). The remaining 74 (72.3%) had other types of sellar lesions such as prolactinomas, somatotropinomas, Rathke's cysts or craniopharyngiomas. Upon admission, 45% of patients with CNFPA presented with both symptoms of pituitary hormone insufficiency and vision impairment, 30% only with pituitary insufficiency, 15% only with symptoms of vision impairment and finally 10% presented with non-specific symptoms such as headache and weakness. Among patients with CNFPA, 14 (33.3%) were revealed to have gonadotropinomas (five men and four women). Pituitary apoplexy was at the initial presentation in six male patients (14.3%) and half of them had gonadotropinomas. Patients with pituitary apoplexy presented bigger lesions ($P<0.05$) and those with a preexisting gonadotropinoma were younger ($P<0.05$) compared to patients without pituitary apoplexy.
Conclusions
CNFPA represent a significant percent of all pituitary adenomas. Pituitary apoplexy is not unusual at initial presentation, especially in younger men with greater sellar lesions.

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P939

Pituitary abscess: a possible cause of hypopituitarism

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Background
Pituitary abscess is a rare but potentially fatal entity. It represents ~0.2–1% of all pituitary lesions. The symptoms are usually nonspecific. The proper interpretation of imaging studies is of extreme importance to the differential diagnoses.
Case
Man, 40 years old, begins complaining of occipital headache (25.04.2011), initially with good response to treatment, without associated symptoms. It became progressively more intense, generalized, and refractory to therapy, associated with photophobia, blurred vision and anorexia. No other neurological, respiratory symptoms or fever were present. There was no previous history of sinusitis or migraine. At the emergency department (ED) (30.04.2011) a cerebral CT was performed with no significant alterations. Because persistence of the symptoms a MRI was performed revealing an apparent enlargement of the adenohypophysis and sphenoid sinusitis. Analgesic therapy was optimized without improvement. The patient was again evaluated at the ED (04.05.2011), the MRI was repeated revealing sphenoid sinusitis and hypophysitis with possible intra pituitary abscess. Analytical study showed increased inflammatory markers. Therapy with

prednisolone, ceftriaxone and metronidazole was started and left sphenoidectomy was performed in 06.05.2011. Pituitary function was evaluated 5 days after steroids therapy revealing low cortisol and ACTH (supression), total testosterone 0.81 ng/ml (2.8–8.0) with normal FSH (1.5–12.4) and LH (1.7–8.6), prolactin 3.5 ng/ml (4.0–15.2) and thyroid function in lower limit of normal. The patient showed progressive clinical improvement and no symptoms of pituitary hypofunction. Resolution of the pituitary abscess was observed in the MRI. Revaluation of pituitary function, 2 weeks after corticosteroid therapy suspension, revealed normal levels of cortisol, ACTH and testosterone.

Discussion

The diagnosis of pituitary abscess remains a challenge even with the aid of imaging techniques.

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P940

Cushing's disease recurrence after pregnancy

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Introduction

Pregnancy is rare in patients with Cushing's syndrome (CS) because of hypercortisolemia, hyperandrogenemia, and/or hyperprolactinemia. Diagnostic tests for CS have become less reliable due to the physiological changes in the hypothalamo-pituitary adrenal (HPA) axis during gestation. Some of these changes may persist until the 5th *postpartum* week. We report a case of Cushing's disease (CD) that recurred after pregnanacy.

Case presentation

A 32-year-old woman presented with a facial plethora, and menstrual disturbances that have developed over 1 year. Biochemical testing suggested CD. Pituitary MRI demonstrated a macroadenoma extending to suprasellar cysterna with displacement of infundibulum. Transsphenoidal surgery was performed. Subsequent biochemical tests suggested remission and her pituatry MRI was normal. This picture continued to be the same during 2 years of follow up. Then, she became pregnant. She presented to endocrinology outpatient clinic at 8 months of pregnancy. She had normal weight gain compatible with her gestation. Her blood pressure and glucose profiles results were also normal. A healthy male infant with normal weight was delivered. At 8 weeks *postpartum*, she developed facial plethora and began to gain weight. At that time 1 mg dexamethasone supression test result was 3.3 μg/dl and the basal urinary free cortisol (UFC) was normal. Since it's immediate *post-partum* period, we thought that these results still might be persistence of increased maternal (HPA) activity during pregnancy. At 5 months *postpartum*, she continued to gain weight and there was elevated UFC and loss of diurnal variation of cortisol. Plasma ACTH level was 41.5 pg/ml. Her pituitary MRI revealed 6.5×3 mm microadenoma surrounding infundibulum suggesting recurrence. Three months later, her blood pressure began to rise. All clinical and biochemical findings have become overt at that time.

Conclusion

In conclusion, this case illustrates CD can recur after pregnancy and hormonal changes observed during pregnancy make the diagnosis more challenging.

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P941

Clinical presentation, treatment approach and outcomes of patients with prolactinomas in real-life clinical practice: a single center experience

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Introduction

Prolactin (PRL) -secreting adenomas are about 40% of all pituitary adenomas. Their clinical symptoms are mainly related to development of secondary hypogonadism and/or tumor mass effects. Dopamine agonists (DA) are first-line drugs for prolactinoma patients due to their effectiveness in normalizing PRL levels and shrinking tumour mass. Surgery is an option for DA failure or tumour mass effects.

Objective

To assess clinical characteristics and treatment outcomes of patients with prolactinomas.

Methods

Retrospective review of the clinical records of 72 patients (22 men and 50 women) with prolactinoma diagnosed from 1984 to 2012 and followed up in our outpatient clinic.

Results

The mean follow-up time was 103.82 ± 80 months(range 7–338). Men was older at diagnosis (40 ± 16 vs 28.72 ± 10.33 years) ($P < 0.001$).The most common clinical symptoms in men were erectile dysfunction (59.1%) and headache (45.5%), whereas in women were oligo-amenorrhea (88%) and galactorrhea (58%). Incidental diagnosis was done in 10 patients (nine men and one woman). Symptoms related to tumour mass effect were more frequent in men ($P < 0.01$). Within the 72 patients, 39 (54.2%) had microadenomas and 33 (45.8%) had macroadenomas. Macroprolactinomas showed higher mean basal serum PRL levels ($P < 0.001$) and were more frequent in men ($P < 0.001$). Sixty-five patients (90.2%) received long-term DA therapy alone and seven patients were treated by pituitary surgery followed by long-term DA therapy. Frequency of surgical removal was higher in men ($P < 0.05$). At the end of follow-up, complete or partial remission (no visible or reduced tumour in CT or MRI and normal PRL levels on/off current DA medication) were achieved in 77.7% of patients. Prevalence of adverse drug effects were lower in cabergoline treated subjects ($P < 0.05$). Seventeen out of 26 patients (65.4%) recurred after DA withdrawal. Recurrence was more frequent in patients with macroadenomas ($P < 0.05$).

Conclusions

In our study, prolactinomas showed different and more aggressive clinical characteristics in men. DA therapy normalized prolactin and reduced tumour size, alone or associated with surgery, in the majority of patients.

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P942

Clinical characteristics of patients with congenital hypopituitarism in advanced age

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Background

Hypopituitarism is considered to be a risk factor for cardiovascular disease and early death in humans. However, some studies showed that most patients with isolated GH deficiency or combined pituitary hormonal deficiency due to gene mutations (PROP 1, GH receptors gene, GH-1 gene) can survive to advanced age.

Aim

To collect clinical data on patients with congenital hypopituitarism older than 50 years identified in the Department of Neuroendocrinology, Belgrade, Serbia between 2005 and 2012.

Patients

Our database contains in total 56 patients with congenital hypopituitarism. Eight of them are older than 50 years (five females and three males, range 53–66 years). Their adult phenotype is typical for childhood onset hypopituitarism, with dwarfism, dry wrinkled skin and lack of sexual development. Their final heights are from 127 to 168 cm and BMI from 16.4 to 31.2 kg/m^2. According to the number of anterior pituitary hormone deficiencies out of eight elderly patients, five have GHD3 while three patients have GHD2. These patients were not treated with GH, started hormone replacement treatment late in adulthood and were not compliant with the replacement therapy. However, their bone mineral density is not reduced. Glucose metabolism is normal in all and only one patient presented with abnormal lipid profile. They did not have any serious diseases during the life-time. Unfortunately one female and one male patient died due to gastrointestinal bleeding of unknown cause. In most presented patients MRI showed pituitary stulk disconnection and small pituitary.

Conclusion

Our data show that patients with congenital hypopituitarism in advanced age can still have a healthy life style with the abscence of cancer, metabolic and cardiovascular diseases.

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P943

Pituitary abscess after cabergoline treatment of a prolactinoma in a 56 year old male: case report and review of the literature

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Objective
Abscesses in pituitary adenomas are rare. Continuous progression and haematogenous spread of infections are considered the main pathogenic mechanisms. A standard therapeutic regimen does not exist.
Methods
We report on the case of a 56-year-old male who had been treated with cabergoline for a macroprolactinoma for 4.5 years. The patient presented to our emergency room with sudden deterioration of visual acuity and right oculomotor paresis. A pituitary abscess was identified in MRI, urgently evacuated transsphenoidally and then treated with i.v. antibiotics. We provide clinical, histopathological and MRI findings as well as a follow-up at 21 months after surgery. We compare our case to other cases from the literature and, considering the evidence in rodents, discuss potential pathogenic mechanisms with special regard to immunodeficiency.
Results
The favourable result we observed so far as well as the cases reported in the literature warrant to consider urgent transsphenoidal evacuation followed by i.v. administration of antibiotics as treatment option of choice in pituitary abscesses. Immunodeficiency due to the mass effect of a macroprolactinoma with subsequent suppression of GH secretion and decreased IGF1 release or due to suppression of prolactin-triggered immunomodulation as a side effect of treatment with dopamine agonists have to be taken into consideration as potentially pathogenic mechanisms.
Conclusions
Although pituitary abscesses are rare, neurosurgeons, endocrinologists and ophthalmologists should be aware of this life-threatening entity. Regular follow-up MRI scans in patients treated with dopamine agonists for a prolactinoma are warranted to timely detect signs of potential inflammation. Urgent transsphenoidal evacuation followed by i.v. administration of antibiotics should be considered as treatment option of choice in pituitary abscesses. The function of the immune system in patients with prolactinoma and treated with dopamine agonists should be a matter of further investigations.

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P944

Tuberous sclerosis: an uncommon cause of hyperprolactinemia

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Objective
To report a case of tuberous sclerosis presenting with hyperprolactinemia.
Methods
Clinical, laboratory and radiographic data are reported on a 26-year-old female presenting with galactorrhea and menstrual irregularities.
Case report
A 26-year-old female with no premorbidities presented with complaints of galactorrhea for the past 10 days and menstrual irregularities over the past 6 months. Galactorrhea was spontaneous. Her last childbirth 4 years ago was uneventful. She had no head ache, vomiting, and visual impairment. She denied any history of substance abuse, drug intake hypothyroidism, chronic liver or kidney diseases, and epilepsy. She was a well nourished female with mild pallor, tiny nodule on face, subungual fibroma in hands. There was spontaneous galactorrhea and mildly tender breasts without any signs of inflammation. Systemic examination was entirely normal with normal IQ. Ophthalmologic evaluation revealed white disk shaped retinal hamartoma. Routine laboratory investigations including renal and liver function tests, thyroid profile were normal. Serum prolactin was 85 ng/ml with FSH-4.66 and LH-4.21 mIU/ml. Tests for evaluation of other anterior pituitary hormones were normal. Abdominal and pelvic ultrasound revealed no abnormality. Chest X ray showed bilateral interstitial infiltrates. Echocardiogram of heart was normal. Computed tomography (CT) scan revealed multiple intracerebral calcifications. These calcified lesions/ subependymal hamartomas are seen along the lateral surface of the lateral ventricles giving rise to characteristic candle dripping appearance. Magnetic resonance imaging (MRI) of the brain ruled out the presence of any pituitary mass. The combined clinical scenario along with the radiologic findings leads to the diagnosis of TSC with hyperprolactinemia. Patient was prescribed cabergoline 0.5 mg twice daily which resulted in amelioration of galactorrhea and regularization of menses.
Discussion
Tuberous sclerosis (TSC) is a multi system genetic disorder which infrequently affects the endocrine system. Cushing's disease, hypoglycemia secondary to insulinomas, precocious puberty, thyrotoxicosis, hypercalcemia secondary to parathyroid adenomas, hyperprolactinemia and acromegaly have all been reported in TSC patients. The circulating prolactin of our patient may be of pituitary origin or may possibly be secreted ectopically by a hamartoma.
Conclusion
TSC patients develop hormone secreting tumors involving the neuroendocrine system.

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P945

Hemiparesis and hemiplegia as clinical presentation in subjects with pituitary tumours

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Introduction
Pituitary tumours are known to cause headaches and visual troubles by compressing pituitary adjacent structures. Pressure on more distant structures is exceptional. In very large tumours such as prolactinomas, mixed adenomas or craniopharyngiomas, hydrocephalus, convulsions, memory troubles and even unconsciousness can be observed, but to our best knowledge motor deficits, such as hemiplegia or hemi paresis, are rarely reported as clinical presentations in pituitary tumours. Our aim is to report four pituitary tumours with hemi paresis or hemiplegia in order to emphasis the different mechanisms of such neurological troubles.
Case reports
Two men aged 30 and 41, a woman of 26 years old, and a baby aged 5 months were sent for progressive or acute hemi paresis/hemiplegia related to pituitary tumours. In these cases pituitary tumours were all giant (height $>$ 4 cm), invasive (invasion of cavernous sinuses), and compressing distant cerebral structures. After medical treatment, the neurological deficits resolved in subjects with prolactinomas. Unfortunately, the 4th case with craniopharyngioma and active hydrocephalus worsened, and then died.
Conclusion
Although hemi paresis and hemiplegia are very rare clinical presentations in people with pituitary tumours; this aetiology should be kept in mind, as motor deficits can disappear after adequate treatment of pituitary tumours, especially large prolactinomas, as in the reported cases.

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P946

Relation with carotid intima media thickness and procalcitonin in acromegalic patients

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Introduction
Acromegaly is a rare, insidious, and potentially life-threatening condition. Patients with acromegaly have an increased morbidity and mortality for cardiovascular diseases. However, data on coronary heart disease and atherosclerosis are controversial in patients with acromegaly. Measurement of carotid intima media thickness (cIMT) with carotid doppler ultrasonography is a useful determiner of early atherosclerosis. Procalcitonin is a 116 amino acid peptide with a sequence identical to that of the pro-hormone of calcitonin, but procalcitonin it has no known hormonal activity. Possible relation with high procalcitonin level in acromegaly has been reported.
We aimed to evaluate the any relation with cIMT and procalcitonin levels in acromegaly.

Methods
In this study, totally 37 patient (16 patient with cIMT <0.8; 21 patient with cIMT ≥ 0.8) were used. Thirty age and sex matched healthy controls were included. We measured cIMT and procalcitonin levels. The cIMT measurements were made at the follow-up visit subsequent to when blood samples were obtained. B-mode ultrasonography of the left and right common and internal carotid arteries was performed by a specially trained radiology technician. The common cIMT was calculated as the mean of the left and right measurements and cIMT >0.8 were evaluated as increased thickness.
Results
We observed higher procalcitonin levels in acromegalic patient with cIMT ≥ 0.8 compared to acromegalic patient with cIMT <0.8 and results were statistically significant ($P<0.05$).
Conclusion
Our results showed that procalcitonin can be useful for the assessment of premature atherosclerosis in acromegaly.

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P947

The role of octreotide LAR treatment on BMI in patients with acromegaly

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Objectives
It is known that the obesity and insulin resistance are an integral part of acromegaly. The aim of study was to evaluate the role of octreotide LAR treatment on BMI in patients with acromegaly.
Methods
Sixteen patients with acromegaly diagnosed at Endocrinology Clinic in Sarajevo (10 females and 6 males, mean age 53.4 ± 6.3 years, age range 38–65 years, six patients with microadenoma and 10 patients with macroadenoma) were treated with octreotide. Follow-up period was 3 years (2009–2013). Nine patients were treated with surgical and octreotide treatment. One patient was treated with surgical, octreotide and gamma-knife treatment and six patients were treated only with octreotide LAR. Five patients were diabetics. Anthropometric measurements (including height, weight, BMI and waist), concentration of human GH (hGH), IGF1, CRP, blood glucose, basal insulin and lipid profile were evaluated before treatment and every 6 months during follow-up period of 3 years, while magnetic resonance imaging (MRI) was taken before the treatment and every year during treatment. Thirteen patients received octreotide 30 mg/28 days, one patient received 20 mg and other two 60 mg/28 days.
Results
Octreotide LAR significantly reduced GH (50.13 ± 22.44 vs 2.11 ± 0.56 ng/ml), IGF1 (749.54 ± 112.48 vs 337.33 ± 83.54 ng/ml), adenoma size and CRP (4.56 ± 1.34 vs 2.34 ± 1.01 mg/l) and non-significantly reduced level of basal insulin and cholesterol. During follow-up period octreotide LAR treatment significantly reduced dosage of exogenous insulin at diabetics (-43%). Regression analyses showed inverse association of octreotide treatment and BMI ($P<0.01$).
Conclusions
Treatment with octreotide LAR in acromegaly significantly reduced BMI, GH, IGF1 and CRP. As well this treatment reduced dosage of exogenous insulin at acromegalic patients with diabetes.

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P948

Effect of somatotropin and IGF1 secretion on glucose metabolism: diabetic ketoacidosis as first manifestation of acromegaly

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Insulin and somatotropin (GH) have opposite effects in glucose metabolism. GH increases the production of glucose through lypolisis and inhibits hepatic and peripheric neoglucogenesis induced by insulin. When insulin secretion is insufficient to overcome insulin resistance, intolerance to glucose or diabetes appears. Drugs used in the treatment of acromegaly also influence glucose metabolism: SSA decrease insulin secretion and pegvisomant has the opposite effect.

The authors present the cases of three male patients, mean age 23.9 years, who were diagnosed with diabetic ketoacidosis (DKA) as first manifestation of acromegaly. No personal or family history of diabetes. All had marked physical features of acromegaly and macroadenomas on MRI.
In two patients, initial HbA1c/GH/IGF1 were 9.3%; 155 ng/ml; 458 ng/ml and 11.8%; 229 ng/ml; 1577 ng/ml. They were initially treated with insulin (maximal doses of 0.26 and 1.28 U/kg per day) and metformin 2 g and 3 g/day. No predisposing factor for DKA was identified. They had surgery with partial resection of the adenoma and began treatment with SSA, with marked improvement in glycemic control and progressive reduction of insulin dose, which was suspended 5 and 7 months later. They maintain increased levels of GH and IGF1 and will be treated with surgery and radiosurgery, respectively.
The third patient presented with pituitary apoplexia and hypopituitarism at the moment of diagnosis (HbA1c 9.3%; IGF1 38.8 ng/ml). After resolution of DKA, he didn't temporarily need treatment with anti-diabetic medication. However, 2 months later, he had to start treatment with insulin (HbA1c 12%; IGF1 30 ng/ml). He is currently also being treated with levothyroxine and prednisolone and awaits for surgery.
DKA due to relative/absolute insulin deficit is rare as first manifestation of acromegaly (1%). In our series, it represented 6.4% ($n=47$). The effects of GH and IGF1 on glucose metabolism are complex and could be reversible with the normalization or reduction of GH levels.

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P949

Prolactinoma: does the size affect the signs?

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Introduction
Prolactinoma is the commonest pituitary adenoma. The aim of this study was to compare clinical, biological and radiological characteristics in microprolactinoma and macroprolactinoma.
Patients
This retrospective study included 33 patients with prolactinoma. The tumor was a microadenoma (<1 cm) in 27.3% ($n=9$) and a macroadenoma in 72.7% ($n=24$) of cases.
Results
Patients were female in 84.8% of cases ($n=28$). Mean age at diagnosis was 37 years (ext: 20–78). Diagnostic circumstances were symptoms related to hyperprolactinemia in 54.5% ($n=18$), and tumoral syndrome in 45.5% ($n=15$) of cases. The first complaint was tumoral syndrome in 30.4% ($n=7$) of patients. Mean progression period was 44.38 months (ext: 0.2–264). Mean serum prolactin level was 4488.35 ng/ml (ext: 31.9–76 915). Mean size of adenoma on MRI was 20.93 mm (ext: 5–60). Comparing microadenoma and macroadenoma revealed that macroadenoma was associated with a higher diagnostic age (39.29 vs 30.89 years) but a shorter progression period (39.381 vs 62.4 months). Tumoral syndrome was present in 58% of macroprolactinomas and in only one case of microprolactinoma while signs of hyperprolactinemia were observed in respectively 58 and 89% of cases. Mean prolactinemia was 5956.40 and 84.18 ng/ml respectively in macroprolactinoma and microprolactinoma. In macroprolactinoma, hemorrhage signs on MRI were present in 37% ($n=9$), cavernous sinus extension in 29% ($n=7$), sphenoidal extension in 17% ($n=4$) and chiasmatic invasion in 12.5% of cases; while a single case of sphenoidal invasion was found in microprolactinomas. Thyreotropic and corticotropic deficiencies were found in 17 and 12.5% of macroprolactinomas.
Conclusion
These results are particular by the relative high frequency of macroprolactinoma. Tumoral syndrome is the commonest symptom in macroprolactinoma. A shorter time to diagnosis in macroprolactinoma suggests the important impact of this complaint on patients motivating them to consult.

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P950

Diagnosis and treatment of a population of acromegalic patients

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Introduction
Acromegaly is a chronic disease caused by GH hypersecretion resulting in increased IGF1 levels. The actions of these hormones result into a broad spectrum of clinical manifestations.
Objective
To evaluate clinical and analytical parameters, imaging, and treatment outcome in a population of acromegalic patients.
Methods
Retrospective study of acromegalic patients diagnosed between 1982 and 2011. Results are presented as mean $\pm$ S.D.
Results
We included 98 patients (68 women) with a mean age at diagnosis of 45.4 $\pm$ 14.6 years and a diagnostic delay of 6.8 $\pm$ 5.3 years. Acral enlargement was the chief complaint (24.5%), followed by headache (18.4%) and maxillofacial changes (14.2%). The most common comorbidities were hypertension (36.7%), carpal tunnel syndrome (34.7%), sleep apnea (26.5%), thyroid nodules (25.5%), impaired glucose tolerance (22.4%) and diabetes (17.3%). Most women (68.6%) reported menstrual disturbances. The majority of tumors were macroadenomas (77.6%) at the time of diagnosis and 36.7% secreted both prolactin and GH. The mean basal GH at diagnosis was 28.5 ng/ml and mean nadir GH achieved during OGTT was 20.8 ng/ml. Gonadotropins deficit was the most common deficit (15 patients), two patients had pan-hypopituitarism, two had combined TSH and gonadotropins deficit and one TSH isolated deficit. The majority of patients (90.8%) were submitted to surgery, mainly by transsphenoidal approach, and cure (biochemical normalization and no recurrence during follow-up) was achieved in 37.1% patients.
Discussion
Although several studies suggest that acromegaly is diagnosed in approximately equal numbers of men and women, in this population there was a higher prevalence of the disease in women, which may reflect a greater concern with body image. The mean age at diagnosis, clinical manifestations and tumor size are similar to those described in other epidemiological studies. We consider a cure rate acceptable considering criteria applied.

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P951

Syndrome of inappropriate antidiuresis due to low dose hydrochlorothiazide use in a patient after transsphenoidal surgery for pituitary adenoma

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Introduction
Hyponatriemia is one of severe postoperative water-electrolyte disturbances. This condition is usually caused by syndrome of inappropriate secretion of antidiuretic hormone or cerebral salt-wasting syndrome which require different treatment approaches.
Case presentation
A 61-year-old woman was referred to tertiary care center with non-functioning pituitary macroadenoma. The patient had been suffering from headaches for 4 later years and had been treated with antihypertensive therapy that included ACE inhibitors and 12.5 mg of hydrochlorothiazide for several years. Macroadenoma was detected 2 years ago and in 2012 the MRI showed rapid suprasellar tumor growth.
At presentation
TSH 0.43 mIU/ml (0.25–3.5), fT_4 15.31 pmol/l (9–20), ACTH 22.21 mg/ml (7–66), cortisol 436.6 nmol/l (123–626), FSH 19.6 U/l, LH 24.3 U/l, prolactin 214.1 mU/l (90–540), IGF1 76.67 (54–210). MRI revealed pituitary adenoma $16\times20\times13$ mm with supra- and parasellar extensions. Intraoperatively the tumor occupied all of the sella space, infiltrated the pituitary and was closely attached to the back wall of the sella turcica.
The postsurgical period starting from 2d p.o. day was complicated by antidiuresis (fluid intake 2000–2700 ml, diuresis 1000–1200 ml), neurological disturbances (fatigue, vertigo, shaky walk). Postoperative hypothyroidism and hypocortisolism were excluded. The lab tests were only notable for hyponatriemia – 126 mmol/l (135–146). The i.v. therapy with normal saline (3% was not available), oral fluid intake restriction, increased oral salt intake, fludrocortisone 0.1 mg a day didn't correct the clinical situation with variable electrolyte values in subsequent 4 days (Na 127–117 mmol/l, K 4.0–3.6 mmol/l, Cl 93–80 mmol/l, plasma osmolality 0.274–0.243 Osm/kg with inappropriately high urine osmolality 0.541–0.470 Osm/kg). The patient's condition improved and Na normalization (139 mmol/l) occurred only after discontinuation of hydrochlorothiazide. After that fludrocortisone was withheld and no water-electrolyte disturbances were detected at follow-up control. Thereby, this clinical situation was considerate as thiazide-induced antidiuresis.
Conclusions
The case of our patient raises the problem of hard differential diagnosis and treatment of hyponatriemia in patients undergoing transsphenoidal surgery.

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P952

An acromegalic patient with giant tumor

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Introduction
Acromegaly is a disease caused by excessive secretion of GH. The cause of acromegaly is GH secreting pituitary adenoam in 99% of the cases. It is estimated that 5% of pituitary adenomas become invasive and may grow to gigantic sizes (>4 cm in diameter). Here, we present a 31 years old acromegalic woman with a delayed diagnosis of a giant invasive pituiatary adenoma.
Case report
A 31-year-old woman admitted to our hospital for oligomenorhea and loss of vision. She had a history of infertility for 10 years. She received IVF five times. Her FSH was 0.43 (3.5–12.5) mU/ml, LH was 0.07 (2.4–12.6) mU/ml, prolactin was 27 ng/ml (4.7–23) and IGF1 was 984 ng/ml (116–307 for her age). Her nadir GH level during a 75 g oral glucose tolerence test was 14.8 mg/dl. Pituitary magnetic resonance imaging revealed a $72\times47\times68$ mm macroadenoma with necrotic componenets which invades suprasellar region and sphenoid sinus and destructs sphenoid bone. Despite a giant macroadenoma her thyrotrophes and corticotrophes were not affected. She had normal thyroid and adrenal fucntions. She had transcranial removal of the adenoma and histopathological diagnosis was eosinophilic somatotroph adenoma. Immunhistochemical staining was positive for GH. Postoperatively, she had severe hypernatremia and she was followed in intensive care unit. She died in her second postoperative day with severe electrolyte imbalance.
Conclusions
Delayed diagnosis of acromegaly may lead to growth of a giant macroadenoma which then increases the likelihood of morbidity, postoperative complications and mortality.

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P953

Hyperprolactinemia in women

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The aim of this study was to determine the structure of the primary incidence of hyperprolactinemia among the female population of the city during 2011. 114 medical cards of women with hyperprolactinemia were researched and analyzed. It was determined that the mean age of the case detection was 28.9 years ($\sigma=8.2$). The main clinical features were the disturbances of menstrual period (41.2%), infertility (26.3%), galactorrhea (21.9%) and the changes of the body weight (7.9%). Among the disturbances of menstrual period were amenorrhea (40%) and irregular menstruation (60%). The majority (73%) of women with infertility have no any other clinical features. By the end of 2011 the pituitary MRI scan was performed in 81 women with hyperprolactinemia. The results of the pituitary MRI showed that 72% (58) of women with hyperprolactinemia had microadenomas,

6% (5) had probable signs of microadenomas, 22% (18) had no signs of microadenoma. 63% (20) of hyperprolactinemic women with the disturbances of menstrual period had microadenomas, 9% (3) had probable signs of microadenomas, 28% (9) had no signs of microadenoma. 72% (18) of infertile women with hyperprolactinemia had microadenomas, 4% (1) had probable signs of microadenomas, 24% (9) had no signs of microadenoma. 100% (17) hyperprolactinemic women with galactorrhea had microadenomas.
The results show that it's necessary to test a serum prolactin of infertile women. The pituitary MRI in hyperprolactinemic women is recommended to diagnose a microadenoma.

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P954

Radiotherapy in the management of pituitary functioning adenomas: a single-center experience

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Introduction
Radiation therapy is an effective treatment for relapsing or recurrent pituitary functioning adenomas, providing tumor volume control and hormone secretion normalization. However, there are several secondary effects to consider.
Aims
To assess the efficacy of radiotherapy in the management of patients with acromegaly and Cushing's disease (CD) treated in our Department, and the prevalence of radiation secondary effects.
Methods
Retrospective analysis of clinical data, hormonal and radiological parameters of all the patients with acromegaly and CD submitted to radiotherapy from 1989 until present time.
Results
Total of 28 patients, 7♂ (25%) and 21♀ (75%); 75% with acromegaly and 25% with CD. Fractionated external beam radiotherapy was performed in 78.6%, GammaKnife radiosurgery in 14.3% and fractionated stereotactic conformal radiotherapy in 7.1%.
In the acromegaly group, all the patients had previous pituitary surgery. Thirteen patients (61.9%) had also therapy with somatostatin analogs that were discontinued after radiotherapy in 28.6% of the cases. Remission was achieved in 42.9% of the patients after 109.5 ± 68.9 months. Tumor volume reduction was visible in 61.9% of the cases after 85.2 ± 62.4 months.
In the CD group, six patients (85.7%) were previously submitted to surgery. All of them were under steroid synthesis inhibitors for 48.3 ± 66.6 months, which were stopped after radiotherapy in every patient. Remission was achieved in four patients (57.1%), after 70 ± 61.1 months. There was tumor shrinkage in six cases (85.8%), after 67 ± 61.8 months.
The reported secondary effects were: stroke in one patient, meningioma in one, epilepsy in two and dementia in one. Of the 11 (39.3%) patients with previous hypopituitarism, 4 (36.4%) worsened the number of deficits. There was *de novo* hypopituitarism in nine patients (32.1%).
Conclusion
In this sample, radiotherapy was effective in the normalization of hormonal hypersecretion (remission) in 46.4% of the patients, and tumor volume control (decrease of the tumor residue) in 67.9%. The most prevalent secondary effect was hypopituitarism (68.5%).

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P955

Atypical McCune–Albright syndrome associated with GH secreting pituitary adenoma

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McCune–Albright syndrome (MAS) is characterized by fibrous dysplasia (FD), cutaneous cafe-au-lait pigmentation and autonomous hyper-secretory endocrinopathies. Association of acromegaly with MAS is very rare. We present a case of a 35-year-old men with MAS, diagnosed with poly-ostotic fibrous dysplasia at the age of 12, no history of premature puberty, with GH secreting cystic macroadenoma $40 \times 35 \times 45$ mm big and clinical picture of acromegaly, hypopituitarism and bilateral hemianopsia. FD was diagnosed based on the clinical picture, radiological findings and bone scan.
Base values of the hormones were: GH 16.6 ng/ml with absent supression OGGT, IGF1 >1100 ng/ml, IGFBP $=9800$ mg/ml, PRL >470 mg/ml.
Our patient was treated with somatostatin analog, Octreotide 3×0.1 mg for 2 months prior to transfenoidal surgery. Due to residual and activity of acromegaly, the patient was treated with external irradiation and dopamine agonist Bromergon until normal values of GH/IGF1 were achieved. One year after the surgery, FD of cranofacial bones caused fascial assymetry, ptosis of the right eyelid, strabismud, sight imparements. After Craniotomia frontotemporalis dex. Decompresion.optici dex. were performed, the symptoms disappeared. The pathohistological findings showed Osteoma (FD) of the scalp in the right occipital region. The patient is on substitution therapy with L-thyroxine, testosterone and bisphophonate.
As conclusion, primary treatment of large pituitary mass is surgery. Nonsurgical treatment, in inoperable patients due to bony involvement of the skull are somatostatin analogs, radiotherapy and dopamine agonists at maximal doses.
Key words: McCune–Albright syndrome, acromegaly.

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P956

Hyponatremia as a first sign of panhypopituitarism: empty sella

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Introduction
Hyponatremia is the most common disorder of electrolytes encountered in clinical practice, occurring in up to 15–30% of both acutely and chronically hospitalized patients. Although most cases are mild and relatively asymptomatic, it is important clinically because: i) acute severe hyponatremia can cause substantial morbidity and mortality, ii) mortality is higher in patients with hyponatremia who have a wide range of underlying diseases and iii) overly rapid correction of chronic hyponatremia can cause severe neurologic deficits and death.
Hyponatremia as the presenting manifestation of empty sella syndrome is rare. The term empty sella makes reference to the herniation of the subarachnoid space within the sella in patients with no history of pituitary tumor, surgery or radiotherapy. Although it is not usually associated with endocrine abnormalities, different degrees of hypopituitarism and mild hyperprolactinemia have been reported. Its clinical presentation resembles that of the syndrome of inappropriate antidiuretic hormone (ADH) secretion, but fluid restriction alone is unable to correct this problem. The cause of ADH secretion in hyponatremia associated with hypopituitarism is related to adrenocortical deficiency. The glucocorticoid deficit is not an osmotic, but a physiological stimulus for ADH secretion. Glucocorticoids have been shown to reverse the impaired water diuresis of this disorder by increasing the renal excretion of solutefree water. Glucocorticoid substitution is the mainstay treatment in this setting, but there are no practical guidelines for optimal glucocorticoid correction. In reviewing the literature, the dosage of hormone substitution has varied. Frequent monitoring of natremia is necessary to prevent neurologic deficits and myelinolysis.
Case report
A 38-year-old patient was admitted to our Department in April/May 2011 due to dyspepsia, nauzea and vomiting. Patient had a history of treatment with NSA (nimesulid) 14 day before admission, after falling down he complained of the pain in the right thorax. On the date of admission he had following laboratory findings: severe hyponatremia 118.8 (reference range RR 135–145 mmol/l), low serum osmolality 240 (275–295 mOsm/kg), urine osmolality 547 (50–1400 mmol/kg), uTSH 2.92 (0.3–4.2 μIU/ml), serum cortisol level at 0900 h was 616 (500 nmol/l and more), mild anaemia Hb 117 (130–180 g/l). He suffered from asthma and had meningitis in 1994. He had no liver and renal disease and never drank alcohol, he denied any history of using diuretics. His weight was 71 kg, his height was 171 cm, pulse rate 58 beats/min, respiratory rate 19/min, blood pressure 110/70 mmHg, temperature 36.7 °C. He was clinically euvolemic. Patient underwent gastroscopy with conclusion: minimal antrumgastritis and dysmotility like dyspepsia. Colonoscopy was without pathologic findings. CT of thorax and abdomen was without traumatic or other pathological changes, as well as CT of brain. After water restriction the serum natrium level normalised and patient was discharged. According to baseline laboratory findings, thyroid and severe adrenocortical deficiency was excluded. The follow up laboratory findings in

June 2011 were: Na 134.5 (135–145 mmol/l), K 4.69 (3.6–5.3 mmol/l), ACTH 18 (7.2–63.3 pg/ml), IGF1 46.8 (109–284 ng/ml), low level of gonadotropins FSH 2.68 IU/l, LH 1.31 IU/l, very low serum testosteron 0.89 (8.69–29 nmol/l), uTSH 2.92 (0.3–4.2 µIU/ml), fT_4 8.7 (12–22 pmol/l), aTPO 5.43 (5–34 IU/ml), aTG <10 (10–115 IU/ml), cortisol 298 (more than 500 nmol/l), prolactin 15.6 (4.0–15.2 ng/ml), MRI of hypophysis shown empty sella. We suggested panhypopituitarism – ACTH deficiency, TSH deficiency, gonadotropin deficiency and GH deficiency. Patient was tested at 5th Department of Internal Medicine University Hospital in Bratislava, where our suspicion was confirmed. Patient was started treatment with hydrocortison in daily dose 15 mg, levothyroxin in daily dose 75 µg, testosteron replacement therapy every 2–3 weeks and growth hormone replacement terapy with complete restitution of laboratory findings. Nowadays patient is feeling healthy and using all recommended medication.
Conclusion
Hyponatremia is the most common electrolyte disorder. Its frequency is higher in females, the elderly, and in patients who are hospitalized. Complete differential diagnosis including endocrinology laboratory and imaging techniques is necessary to obtain a correct diagnosis and following treatment.

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P957

Somatotrop adenomas: comparison between men and women in algerian population

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Introduction
Pituitary adenomas in general are deemed to be more aggressive in males. Our aim is to analyze somatotrop adenomas (SA) characteristics and neurological complications. In the second time we will compare male and female cases.
Subjects and methods
In this retro-and prospective study, 112 pure and mixed SA diagnosed between 1980 and 2012 were analyzed. They all had hormonal assessment (GH before and after OGTT ± IGF1, PRL, cortisol, ACTH, testosterone/E_2, FSH, LH, TSH, FT_4), ophthalmological examination and cerebral CT scan, or MRI or both.
Results
Sex-ratio = 1, mean age 39.11 years (14–60). Paediatric forms = 8.03%. Mean tumour volume = 117.1 mm^3. Giant forms (height $\geq$40 mm) = 10%. Mean GH = 62.3 ng/ml. IGF1 was increased in all tested cases. Cavernous system invasion was observed in 42.15%. Gonadotrop deficit = 44.8%, corticotrop = 22.22% and thyreotrop = 21.59%. Global pituitary insufficiency = 4.58%. Posterior pituitary deficit = 0%. Optic atrophy = 2.9%. We did not observe any severe neurological complications such as hydrocephalus, convulsions, frontal syndrome, meningitis, memory troubles and unconsciousness, but we had some apoplexy.
Sexual comparison showed men were diagnosed later (39.89 years vs 30.33, $P = 0.01$). We did not find any difference in tumour volume and GH rate before and after OGTT, but women had more invasive tumours ($P = 0.03$). Thyreotrop and gonadotrop deficits were more frequent in women (respective $P = 0.016, 0.000075$).
Conclusion
In this study where the sex-ration is equal to one, female cases appeared more aggressive although male cases were diagnosed later. To explain this difference the role of estrogens should be discussed.

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P958

Non functionning pituitary adenomas

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Introduction
Non functioning pituitary adenomas (NFPA) frequency varies from 15 to 30% among all pituitary adenomas. Some authors think they are more aggressive and at risk of pituitary apoplexy. Our aim is to analyze their characters and their complications.
Subjects and methods
It is a retrospective study that takes in account 37 subjects having a NFPA and recruited from 2005 to 2012. Among this group only 13 have been operated on and had immunochemical study. They were all questioned and had clinical and ophthalmological examination. Hormonal assessment and cerebral MRI were also done. We checked for posterior pituitary insufficiency too.
Results
We observed 26 men and 11 women (sex ratio = 0.42), mean age = 50.13 years. Among them 15% were gonadotrop. They consulted for visual troubles in 44%, severe neurological problems in 33%, endocrine problems in 11% and the NFPA was incidentally discovered in 19.4%. They are giant (height $\geq$4 cm) in 21.7% and invading the cavernous sinuses in 45%. Other invasions are: anterior (orbital and/or nasal) = 8%, temporal (6.45%), frontal (6.45%), post (2.7%). Among endocrine complications gonadotrop deficit is observed in 51% and a multiple deficit in 36%. Diabetes insipidus = 2.6%. Thyreo- and corticotrop axis are the most preserved. Optic atrophy ± nerves palsy = 42%, convulsion, apoplexy, hydrocephalus and unconsciousness are observed in 10.8%.
Conclusion
NFPA prevail in male, there are giant in 21% and invasive in 45% which explains numerous complications such as multiple pituitary deficits, severe ophthalmological and life threatening neurological troubles which are observed in a high rate compared to other pituitary adenomas.

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P959

Assessment of bone mineral density in patients with Sheehan's syndrome

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Introduction
Although the cause and effect relationship between the hypophyseal deficiency and osteoporosis is known well, the number of studies concerning bone mineral density (BMD) in patients with Sheehan's syndrome (SS) are scarce. We aimed to investigate the relationship between BMD and deficient hormones in patients with newly-diagnosed Sheehan's syndrome.
Material and methods
Thirty-four patients with SS and age-gender-weight matched 22 controls were included in the study. Demographic data, biochemical hormonal values and BMD of the patients were recorded.
Results
We found osteoporosis in 61.8%, osteopenia in 32.3%, and normal dual energy X-ray absorptiometry (DEXA) findings in 5.9% patients. In the control group 68.2% of individuals were osteopenic and 31.8% of them were normal. The number of osteoporotic patients was found to be higher and BMD values lower in the patient group compared with the control group. There was no relation of DEXA measurements with the period between the last delivery and the initial diagnosis and the estradiol levels. While there was no relation between IGF1 and T and Z scores of femur head but the relation between the of IGF1 and L1–L4 T scores was statistically significant ($P = 0.010$). There was a significant relation between the IGF1 and L1–L4 Z scores ($P = 0.001$).
Conclusion
SS patients had decreased BMD values compared with age-gender-weight adjusted control group. In conclusion SS patients should also be assessed in terms of osteoporosis at the time of diagnosis and then proper treatment should be initiated afterwards.
Key words
Sheehan's syndrome, BMD, hypophyseal deficiency.

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P960

Giant invasive macroprolactinoma 2: case report

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Introduction
Prolactinoma are the most frequent pituitary adenoma, considered benign tumor and well respond to medical treatment, but sometimes, in men prolactinoma may be invasive and aggressive occurring neurological signs.

Case 1
Male 53 years old, went to ophtalmology consulting for progressive vision problems associated with headaches from more than a year but denied. On examination visual field showed poor vision in left eye and temporal defect on right one.
CT scan and MRI showed a large invasive mass in the skull base with destruction of sella turcica and compression of brain parenchyma with hydrocephaly (80 mm).
He was sent in neurosurgery, the first hypothesis chordoma or meningioma.
He had hypogonadism and hypothyroidy clinical signs. The hormonal evaluation revealed an hyperprolactinemia 22 000 ng/ml. treatment with dopamine agonist was begun with anti epileptic drugs.
The evolution prolactin levels stabilized, MRI showed partial reduction of the volume at 1 year (30%) and more than 80% at 4 years.vision problems improve but did not recover. Hypothyroidism is treated.
Case 2
Male 36 years old, went consulting to neurology for progressive headaches, dizziness, time and space disorientation, double vision and recently blindness in right eye. MRI revealed a large invasive lesion extending to the supra sella cistern, the optic chiasma and the brain parenchyma in left temporal lobe 75/60 mm.
He was evaluated in endocrinology, tests revealed an hyperprolactinemia 12 000 ng/ml, hypocortisolism and hypogonadism.
He was don dopamine agonist at 2 months he presented an apoplexy with ophtalmoplegy and convulsion with rapid recovery.
At 1 year partial tumor reduction (50%) and prolactin level stabilized. At 3 year we obtained an empty sella turcica, an improvement of visual problems.
Conclusion the importance of hormonal evaluation in cerebral tumors. the responsiveness of these tumor to dopamine agonists confirm the medical option in prolactinoma even when invasive.

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P961

Pituitary hyperplasia secondary to primary hypothyroidism in a child with mosaic trisomy 21

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Introduction
Pituitary hyperplasia (PH) is relatively a rare condition in children. As in adults, it can be secondary to a long-standing primary hypothyroidism and can sometimes be mistaken with a pituitary macro-adenoma as in the following observation:
Case report
Herein, authors describe a girl aged 10 years old sent by her neurosurgeon for pre operative hormonal exploration of a pituitary macro adenoma measuring 13 mm in height. This one was discovered by cerebral MRI when she consulted for an abnormal gait.
After questioning the parents, we learnt she always had difficulty to learn. She had her period 6 months ago, and she is taking levothyroxine 75 µg for 3 months.
Clinical examination revealed a discreet growth retardation (−1.5 SD/TS). The rest of clinical examination did not find anything abnormal except a particular shape of her eyes that look like Mongols'. The karyotype confirmed our impression as it showed a mosaic form (15%). Thyroid assessment showed increased TSH (8 IU/ml). Thyroid ultrasound demonstrated an hypo-echoic gland. Thyroperoxydase antibodies were increased arguing for an autoimmune origin which is common in patients with Down syndrome. Under 100 µg levothyroxine TSH and MRI were normalized.
Conclusion
The association of primary thyroid deficit with a pituitary lesion argued for a secondary thyrotrophic pituitary adenoma which is relatively rare in children. But, this diagnosis was confirmed in retrospect by MRI that showed a normalization of pituitary size under 100 µg levothyroxine. Pituitary hyperplasia due to puberty or hypothyroidism should be known by neurosurgeons who are not used to this conditions.

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P962

Can apelin level used as a marker of active and inactive acromegaly?

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Introduction
Acromegaly is a rare disease that is most often caused by a GH secreting pituitary tumor. Apelin, a newly discovered peptide known as an endogenous ligand for its receptor (APJ) is a cardiac positive inotropic factor that has also hypotensive effects. The apelin and its receptor functions have not entirely been understood yet. The physiological importance of apelin, in acromegaly remains obscure.
The aim of this study was to investigate the differences of apelin levels in active and inactive acromegalic patients.
Methods
In this study 37 acromegalic patients (20 inactive and 17 active acromegaly) and 30 controls were included to the study. Patients with GH $\leq$ 1 ng/l and IGF1 were in normal levels according to ages and sex were classified as inactive; and patients with GH $>$ 1 ng/l and higher IGF1 levels were classified as active acromegaly. Serum apelin level were measured by ELISA method.
Results
Although slight decrease were observed inactive and active acromegalic patients, we couldn't find any statistically significant differences in apelin levels in inactive and active acromegalic patients, but we observed more than 11-fold higher apelin levels in active acromegalic patients compared to controls and results were statistically significant.
Conclusion
We found high levels of apelin in active acromegalic patients. Apelin levels can not to be used the marker of active acromegaly.

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P963

Pasireotide in treatment of Cushing's disease: our first experience

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Background
Although the excision of ACTH-producing tumors is the principal treatment for Cushing's disease (CD), pharmacologic treatment has a well-established role. As corticotroph adenomas express somatostatin (SST) receptors (SSTRs), pasireotide – a pluripotent somatostatin analogue, acting on four of five SSTRs has a potential role in treatment of CD among various medical agents.
Case report
We report a 39-year-old female with recurrence of Cushing's disease (CD) 9 years after the initial successful pituitary surgery. Medical treatment with ketoconazole has to be stopped due to headache and myalgia, therefore pasireotide at dose 0.6 mg s.c. twice daily was started. Increased serum cortisol (FP) levels with loss of circadian rhythm (779–398–435–627–607 nmol/l at 0800–1600–2000–2400–0800 h respectively), with increased basal urinary free cortisol (UFC) (1767–1848 nmol/day), non-suppressible in 2 mg (FP 231 nmol/l, UFC 358 nmol/day) and non-supressed ACTH levels (45.0 pg/ml) decreased even 4 days after starting the treatment – FP levels 281–156–206–140–300 nmol/l (taken at same times), ACTH 32.7 pg/ml. After 2 months, FP were 517–357–181–360–339 nmol/l, UFC 987.798 nmol/day, ACTH (39.7 pg/ml) was not changed. Parameters of glucose metabolism were changed non-significantly – HbA1c (IFCC) 3.91–4.48% (normal value 2.0–4.2%), mean daily glucose levels 6.47–7.27 mmol/l (normal values 3.50–5.90 mmol/l).
Discussion:
After 2 months of the treatment, we stated good efect of treatment, with decrease of ACTH (12%), FP (basal 34%, midnight 43%, average 36%), and UFC levels (49%). Patient tolerated treatment with pasireotide well, but suffered from mild persistent diarrhea as an adverse effect of the treatment. Diarrhea is potential rare side effect of SST-analogs and was also recorded as the most frequent side effect of pasireotide in pilot study by Colao *et al.* (*NEJM* 2012).

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P964

Corticotroph adenoma and fertility

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Introduction
The occurrence of a pregnancy in a woman with a corticotroph adenoma is rare, <25 observations have been reported.
Maternal complications are especially hypertension (60–75%) gestational diabetes (25%) and preeclampsia in 10%.
Fetal complications are spontaneous miscarriage, preterm birth and intrauterine growth retardation.
Observation
We report a case of a 30 years old female followed for primary subfertility. The diagnosis of Cushing's disease is withheld to: obesity, amenorrhea, hypertension and osteoporosis

- Cortisol at midnight = 712 nmol/l
- Cortisol at 0800 h = 809.4 nmol/l ACTH = 9 pg/ml
- Low braking: negative
- Strong braking: positive
- Pituitary MRI: microadenoma 6.4 mm.

Under anti-cortisol arrested 2 weeks later for liver toxicity. The resection of microadenoma was delayed because he was 6 weeks' pregnant.
Noting a gestational diabetes than the 24^{e} week. Delivery term.
After 2 years of remission (post resection)a 2nd spontaneous pregnancy with abortion at 12 weeks.
Conclusion
The impact of secretions ACTH of pregnancy requires a multidisciplinary support.

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P965

Central hypothyroidism and adjusted thyroxine dose study (CHATS): impact of increasing free thyroxine levels in patients with hypopituitarism

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Introduction
Patients with pituitary deficiencies suffer from impaired quality of life regardless of substitution therapy with hydrocortisone, thyroxine (T_4), sex hormones or GH. Central hypothyroidism (CH) is difficult to diagnose and treat because symptoms are non-specific and TSH-levels cannot be used for assessment. There is no consensus for the fT_4-goal of thyroxine-replacement in patients with CH.
Aim
To determine the impact of increased fT_4 on quality of life in patients with hypopituitarism.
Methods
Randomized, double-blind, placebo-controlled trial of additional T_4-supplementation. 40 patients (age 20–70 years) with hypopituitarism and fT_4-levels in the lowest third of normal reference range were included. Patients received placebo or T_4-titration aiming for fT_4 levels in the upper third of reference range, irrespective of TSH values. Total study duration 42 weeks (24 weeks dose adjustment, 18 weeks stable dose). Quality of life assessments (QoL) using four questionnaires (SF-36v2, PGWBS, EQ-5D, AGHDA) at baseline and end of study. Statistics were performed using an analysis of covariance.
Results
The increase in fT_4-values in the treatment group did not translate into significant changes in vitality score as assessed by SF-36v2 (estimated treatment effect 4.65 (95% CI -7.86, 17.15) or general health score (estimated treatment effect is 1.57 (-8.19, 11.33)), nor in any of the other questionnaires (PGWBS -1.11 (-8.80, 6.58); AGHD 0.88 (-2.77, 4.53); EQ5D-VAS -4.40 (-13.45, 4.65)).
Conclusion
The increase of fT_4 to the upper third of normal range did not significantly change the vitality score, general health or quality of life in hypopituitary patients and therefore does not provide support for the commonly used strategy of thyroxine-supplementation to the upper limit of normal. With 40 patients, however, the study may be underpowered to detect small effects. Other explanations for lack of effect include an inappropriately high or low fT_4 goal and further research is required.

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P966

A retrospective-prospective study of the effect of octreotide LAR in acromegalic patients

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Objective
Surgery is considered first-line treatment for pituitary GH secreting macroadenoma. Since surgical removal of the pituitary tumor is subtotal, medical treatment has become the mainstay of acromegaly. The aim of our study was to assess the effect of octreotide LAR (OCT-LAR) therapy in patients with acromegaly.
Patients and methods
Ten acromegalic patients were treated with OCT-LAR 20 mg/28 days. The effect was evaluated after 12 months of treatment. Eight patients received OCT-LAR as adjunctive therapy after surgical debulking, and two as primary treatment. Tumor size was assessed with pituitary magnetic resonance imaging (MRI) before the treatment and after 12 months. Biochemical evaluation was performed at baseline, 6 and 12 months after beginning OCT-LAR. Normalization of IGF1 was considered as biochemical remission of active acromegaly.
Results
Among the evaluated patients five were males and five females, mean age 40.2 ± 8.04 years (age range: 30–50 years). After 12 months of treatment biochemical remission (normalization of IGF1 levels) of acromegaly was achieved in nine patients. Values of IGF1 after 6 and 12 months showed a mean decrease of 55.69 and 71.53% respectively. Average tumor size before treatment and after 12 months was 17.7 and 10.42 mm respectively with an average decrease of 48.18% in size. Also, GH levels were evaluated and showed a mean decrease of 74.57% after 12 months. Along the treatment significant improvements in headache, arthralgia, sweating and soft tissue swelling was attained.
Conclusions
Treatment with OCT-LAR can be considered as effective therapy in achieving biochemical remission and tumor shrinkage in active acromegaly. Given its efficacy and safety it is a treatment of option as primary therapy in patients who are at risk or refuse operative treatment.

Keywords
Acromegaly, octreotide LAR, GH, IGF.
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P967

Craneopahrygioma: a false enemy

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Background
Craneopharygioma is a rare solid or mixed tumor, that arise from remnants of Rathke's pounch. Usually they are in the suprasellar region and very few of them arise from the sella. Bimodal (5–14 years old and 50–75) and slow growth are typical characteristics as well.
Clinical case
A 20 years old boy was refered to endocrine clinic by hypothyroidism, hypotension, and sexual dysfunction. Central hypothyroidism, hypogonadotropic hypogonadism and secundary adrenal insufficiency due to sellar mass was diagnosed. Sellar and suprasellar mixed mass with solid and cystic changes were found and due to its stability for 5 years and because of the clinical presentation was interpreted as a macroadenoma. Sustitutive hormone treatment is prescribed and regular clinical and radiologic follow up is made. For 5 years the mass experimented no changes but in the next evaluation mass enlargament with significant suprasellar extension was seen. Pterional craneotomy are made and a cranepharygioma is diagnosed. Treatment is completed with stereotactic radiotherapy and the patient has a good response so far.
Discussion
Usually calcification in the suprasellar region is seen up to 80% and cyst are present very often (up to 75%). If calcification is not seen, suspection is more

difficult. Bimodal age distribution, typical diabetes insipidus (if pituitary stalk is envolved) visual symtomps and sexual dysfuction are typical clinical presentation in craneopharygioma. Diagnosis is confirmed by pathologic report. In our patient stability on time on MRI, his age and no visual disturbance made us to manage as a macroadenoma with panhypopituitarism. We insited on our diagnosis for years of follow-up with clinical and radiologic stability. We decided to surgery because of quick growth and our misdiagnosis was seen. Craneopharygioma must be distinguished from others sellar mass, but: When must we send to surgery a non typical mass thinking about craneopharyingioma?

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Steroid metabolism and action

P968

Androgenic and estrogenic regulation of skeletal muscle mass and atrophy signaling in male mice

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Background
Hypogonadism in men is associated with low muscle mass and force. Although, androgen replacement is an effective therapy, it can have undesirable side effects. Identifying the androgen regulated molecular mechanisms that increase muscle mass and function could lead to the identification of novel muscle-specific therapeutic targets to improve the clinical outcomes of muscle wasting diseases. In this study, we examined in mice the effects of orchidectomy (after 1, 7 and 30 days), either with or without testosterone or estradiol (E_2) administration, on the muscle-specific ubiquitin protein ligases Atrogin-1, MuRF1 and myostatin gene and protein expression.
Methods
Measurements were made in slow-twitch soleus (SOL), fast-twitch extensor digitorum longus (EDL) and androgen-sensitive levator ani/bulbocavernosus (LA/BC) muscle of male C57BL/6 mice.
Results
Thirty days of orchidectomy was associated with a significant decrease in muscle strength and muscle mass in SOL (13.8%), EDL (12.9%) and LA/BC (63%). These effects were prevented by testosterone treatment. In the LA/BC muscle, Atrogin-1 and MuRF1 mRNA were increased throughout the 30 days of androgen deprivation, which was fully reversed by testosterone administration and partially reversed by E_2 administration. In SOL and EDL, a less pronounced upregulation of both genes was only detectable at the early stages of orchidectomy. Myostatin mRNA levels were upregulated in EDL. However, these changes were not paralleled by changes in the protein levels of Atrogin-1, MuRF1 and myostatin at any time point following orchidectomy. Our data indicate important differences in orchidectomy-induced skeletal muscle atrophy between LA/BC and the locomotor muscles.
Conclusion
These findings also question role of Atrogin-1, MuRF1 and myostatin in this model of muscle atrophy and suggest that other proteolytic targets are responsible for the loss in SOL and EDL muscles mass after androgen deprivation.

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P969

Cross-sex hormone therapy related adverse events: data from a large gender identity unit

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Introduction
Hormonal therapy is part of an established treatment of gender identity disorder, however outcome data regarding mortality and morbidity are scant.
Methods
A specialist center cross-sectional study in 193 transsexual women and 128 transsexual men (mean age 42.5 years) assessing physical health and incidence of possible treatment related adverse events compared to an age-matched female and male control group recruited from a population study in Flanders (1–3 matching). Participants on average used 7.4 years of cross-sex hormone therapy (range: 3 months–49 years) and were 6.6 years since sex reassignment surgery (SRS).
Results
Ten transsexual persons (one transsexual men and nine transsexual women) died during follow-up. Causes of death were suicide, cardiovascular disease ($n=2$), cancer ($n=2$) and suicide ($n=6$). Three percentage of transsexual women ($n=10$) experienced venous thrombosis and/or pulmonary embolism during hormonal therapy. Half of them occurred during the first treatment year ($n=5$), another three at time of SRS. Transsexual women experienced more myocardial infarction (MI) compared to control women ($P=0.001$) but not to control men. Prevalence of cerebrovascular disease (CVD) was higher in transsexual women compared to control men and women ($P=0.05$ and $P=0.02$; respectively). Transsexual men had similar morbidity rates of MI and CVD compared to control population.
Prevalence of type 2 diabetes was higher in both transsexual men and women compared to control women. However, all but one diagnosis in transsexual women were found before start of hormonal therapy suggesting overdiagnosis. We observed an equal prevalence of cancers and HIV infection in transsexual persons compared to control population.
Conclusion
Data of the current study indicate a higher mortality rate in transsexual women and more venous thrombosis, MI and CVD. Transsexual men had similar morbidity rates compared to the general population, apart from a higher incidence of type 2 diabetes.

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P970

Screening for Cushing's syndrome in obese patients

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Background
Cushing's syndrome (CS) is considered to be more frequent among patients with metabolic syndrome. Previous studies have suggested to perform a routine screening for CS in obese patients; however, more recent reports only recommend a case-finding approach in patients with uncontrolled diabetes and hypertension, despite appropriate treatment.
Objective
The aim of this study was to evaluate the prevalence of unsuspected CS in morbidly obese patients in an outpatient's clinic.
Design
Retrospective case-note study.
Patients and methods
We reviewed the medical records of morbidly obese patients referred to our clinic prior to bariatric surgery between January 2001 and December 2011. All patients had a complete medical history including physical examination, and 387 (300 females; mean age 46.5 ± 11.2 years; mean BMI 52.8 ± 27.1 kg/m^2) underwent screening for CS as part or our pre-surgical protocol. As screening for autonomous cortisol secretion, we performed an overnight 1 mg dexamethasone suppression test (DST). Serum cortisol <1.8 μg/dl was the cut-off point for normal suppression.
Results
In the retrospective analysis, prediabetes and diabetes mellitus were observed in 10.20 and 26.40% respectively. In 20 of 387 patients, screening was considered to be abnormal. Seven of these 20 patients had subsequent normal 24 h urinary free cortisol (UFC) levels (150 μg/24 h). In 13 of 20 patients, we repeated an overnight 1 mg DST, on suspicion of failing to take the dexamethasone correctly. Three patients failed to suppress their cortisol levels, two of them were on carbamazepine, which was considered to be a false positive result. The other patient with abnormal UFC levels was diagnosed with CS (0.26%), whose cause was a pituitary microadenoma.
Conclusion
A low proportion of patients with morbid obesity were found to have CS. Our findings suggest that morbidly obese patients should not be routinely screened for CS.

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P971

Role of retinol dehydrogenase 12 (RDH12) in the biosynthesis of 5α-androstane-3β,17β-diol (3β-diol) in the skin

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Using real-time PCR, we found that RDH12 is highly expressed in the skin of Macacafascicularis (mf). It has been known that RDH12 is a retinol dehydrogenase that catalyzes the reduction of retinal into retinol and play an important role in the visual cycle. Indeed, its deficiency is the cause of Leber's congenital amaurosis 3, a genetic disorder characterized by retinal dystrophy affecting both rods and cones. Previously, we have shown that many of the members of the retinol dehydrogenase family, such as RDH1, 5, 11 and 16 could also metabolize 5α-reduced steroids, including DHT, the most potent natural.
In order to determine the possible role of RDH12 in a non-visual cycle and especially in the intracrine metabolisation of DHT in the skin, we perform real-time PCR to quantify its expression levels and *in situ* hybridization to localise its expression in the skin. To determine its activity, we construct expression vectors that express the coding region of human, mf and mouse RDH12 under the control of CMV promoter (pCMV-h,mf,mRDH12), and stably transfect the resulting vectors into HEK-293 cells.
Using cells stably expressed human, mouse and mf RDH12 in culture without addition of co-factor, we show that the enzyme catalyzes effectively the transformation of DHT into 3β-diol and 5α-androstane-3,17-dione (5α-dione) into 5α-androstane-3β-ol-17-one (epi-ADT). *In situ* hybridization shows that RDH12 is highly expressed in mf sebocytes. These data strongly suggest that RDH12 could play an important role in the skin, especially in sebocytes by controlling the intracrine concentration of DHT and retinoic acid levels. RDH12 could thus play an important in acneseborrhea, a disease due to altered sebocyte secretion andis influenced by DHT and retinoic acid. In addition, 3β-diol could also have a yet determined effect in the skin and sebocytes due to its ability to modulate ERβ.

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P972

Differences in adipose tissue lipolysis in critically ill septic patients with and without shock

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Introduction
Critical illness, and sepsis in particular, drives adipose tissue lipolysis up (with triglycerides (TG) being split to free fatty acids (FFA) and glycerol (GLYC)) to meet increased energy demands. Few studies have addressed lipolysis with tissue microdialysis (MD).
Aim
To assess indexes of lipolysis in septic patients with and without shock.
Subjects and methods
The study included 110 men and 73 women (mean age + S.D.: 62 ± 17 years), 66 with SIRS/severe sepsis (SSe) and 117 with septic shock (SSho). All the subjects had a tissue MD catheter placed in femoral adipose tissue upon admission to the ICU. Plasma cholesterol, HDL, LDL, FFA, TG and MD GLYC were measured on days 1 and 6 in the ICU. Analysis was done with repeated measures ANOVA and Pearson's correlation.
Results
Seventy-four patients died. Patients with SSho had lower LDL and higher MD GLYC levels compared to SIRS/SSe on days 1 and 6. Significant positive corellations were found between FFA and MD GLYC in patients with SSho on day 1 and in patients with SIRS/SSe on day 6.
Discussion
Lipolysis was apparently acutely more intense in patients with SSho on day 1 and subsequently subsided whereas it became more pronounced in patients with SIRS/SSe on day 6, verging on chronic critical illness. This dimorphism may provide clues for diversification of nutritional support (carbohydrates vs lipids) in critically ill patients; further studies are warranted.

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P973

UGT2B17 genotype and pharmacokinetic profile of testosterone during substitution therapy in men with hypogonadism

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Introduction
The UGT2B17 gene encodes a glucuronidase which is important for the urinary excretion of testosterone. Recent studies have shown that a common deletion polymorphism in UGT2B17 is strongly associated with significant lower levels of excreted urinary testosterone in men. The objective of this study was to investigate the association of the UGT2B17 gene polymorphism and the dosages of testosterone substitution in male patients with frank hypogonadism.
Material and methods
Two hundred and twenty-eight men treated with Testosterone undecanoate (TU) (Nebido) were retrospectively included. All men were given 1000 mg TU per injection at 0, 6 and 18 weeks. Blood samples were drawn at 0, 2, 6, 12 and 18 weeks after baseline. Sexual hormones were analysed by standard assays, and UGT2B17 genotype was determined by quantitative PCR on isolated DNA from peripheral blood.
Results
Of 13.6% had a homozygote deletions (del/del), 45.0% were heterozygote (del/ins) and 41.2% were homozygotes for the wildtype (ins/ins). Before the 3rd injection (18 weeks), nadir serum testosterone levels did not differ between the three groups in total ($P = 0.065$): median 13.2, 12.7 and 14.0 nmol/l in del/del, del/ins and ins/ins groups, respectively. Estradiol levels tended to be higher in the del/del group (66.5 pmol/l) than in the two other groups (ins/del: 54 pmol/l and ins/ins: 52 pmol/l), although differences did not reach statistical significance. LH, SHBG, the free androgen index (FAI), total cholesterol or haemoglobin did not differ between groups.
At follow-up 2–3 years after initiation of treatment all patients had individual treatment regimes, but no association to genotype was detected.
Conclusion
Nadir serum testosterone levels were not significantly influenced by UGT2B17 genotype in hypogonadal men given standard treatment with TU. Thus, there is no need to consider this genotype as a marker of dosage or interval when initiating testosterone treatment.

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P974

Effects of food components and the ratio of epitestosterone to testosterone on steroid glucuronidation

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The ratio of the glucuronidated epitestosterone (E) and testosterone (T), used in doping tests, is currently under question. The key enzyme involved in E metabolism is UGT2B7 in contrast to T which is mainly metabolised by UGT2B17. Previous results demonstrated that at very high concentrations E acts as a competitive inhibitor to UGT2B17. Similarly, the literature reports competitive inhibition of UGTs by NSAIDS and phenolics that are not substrates of UGT2B17. The aim of this study was to investigate if either T or E glucuronidation is affected by food components (red wine and teas) or by varying ratios of the epimer.
Glucuronidation of T and E was assessed using a novel assay with UGT2B17 supersomes and microsomes pooled separately from males and females. HPLC-UV and LC-MS/MS methods were used to monitor E and T glucuronidation at μg/ml and ng/ml levels. Red wine and tea samples were subjected to HPLC analyses to determine their principal active components (catechins and flavonols) which were assessed for inhibitory activity against UGT2B17 and UGT2B7. The effects of E on T glucuronidation were monitored over the concentration range of 0–200 ng/ml E.
The results show that in supersomes the tea samples and red wine along with their component catechins and flavonols exhibit considerable competitive inhibitory activity against UGT2B17 (e.g. IC_{50} of 64 μM for epigallocatechin gallate). For both microsome and supersome based studies, at 50 ng/ml T, an increase in E resulted in an inhibition of T glucuronidation by up 30% (at T:E 0.5). The use of microsomes also showed variation of testosterone metabolism between males and females as well as varying inhibition levels of T glucuronidation by E. These results highlight the effect of common foods, their components and

epitestosterone on testosterone metabolism at physiological concentrations. These results warrant further studies including *in vivo* investigations.

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P975

Impact of smoking on neuroactive steroids

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Introduction
The dependence on tobacco, diagnosis F 17, is an independent condition in the International classification of diseases and presents one of the most widespread addictions. Chronic smoking can cause impairment of fertility in both sexes. However, only a small number of studies were dedicated to the influence of smoking on levels of steroid hormones and their neuroactive metabolites. Neuroactive steroids modulate the effects of tobacco on central nervous system, and also the severity of tobacco dependence. In our study we compared the levels of neuroactive steroids in men smokers and non-smokers and we looked for a possible predictive marker of success in smoking cessation.
Methods
We examined 76 men before initiating the smoking cessation, after 6 weeks and after 1-year of abstinence. According to the success we divided our samples to three groups, 26 men were successful in smoking cessation, 16 men stopped smoking only for 6 weeks and 34 men did not stop smoking at all. We collected samples from a control group of 20 male non-smokers. We measured basic anthropometric data, levels of steroid hormones and their neuroactive metabolites by GC–MS.The local Ethics Committee approved the study and all patients have signed an informed consent form before taking part in the study.
Results
Comparing smokers and non-smokers, we have found lower levels of testosterone, as well as higher levels of FSH, in smokers. Lower levels of testosterone and some neuroactive metabolites of androgens were associated with failure in smoking cessation.
Conclusion
The levels of testosterone and neuroactive metabolites in men successful in smoking cessation were the closest to the levels in the control group. The greater disorder of steroidogenesis, the less probable the success.
Funding
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P976

Changes of steroidogenesis subject to weight growth

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Introduction
Obesity epidemic has a considerable effect on the health of the population. Obesity is connected with infertility and hypogonadism. It is known that obese men have a low testosterone and higher estradiol level. The assumed mechanism would be increased testosterone aromatization in adipose tissue and a lower SHBG level, which means a decrease of overall testosterone. The aim of this study was to describe the changes of steroidogenesis with obese men.
Methods
We examined a set of 195 men and determined their testosterone (T), dihydrotestosterone (DHT), SHBG, androstendion (A2), DHEA, DHEAS, 17-hydroxyprogesterone (Prog17), 17-hydroxypregnenolone (Preg17), the Prog17/Preg17, A2/DHEA, DHEA/Preg17, A2/Prog17, DHEA/DHEAS, T/A2 and DHT/T ratios were analysed. The set of men was divided into two groups in accordance with the BMI level.
Results
With BMI over 30 men a higher activity of 3β-hydroxysteroid dehydrogenase and lower activity of sulfatase have been proved. The preferred way of steroid production with obese men is thus Δ-4 way. With men the Δ-5 way is typical, on the contrary the Δ-4 way is typical with women.
Conclusion
We have proved a steroidogenesis shift with obese men that accounts for the T and E_2 level change typical with obese men.
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Thyroid (non-cancer)

P977

Determinants of between-subject variation in thyroid hormone status in healthy young men

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Background
Interindividual variation in thyroid hormone concentrations is greater than intraindividual variation, suggesting that individuals have different set-points for pituitary–thyroid axis function. Variation in thyroid hormone levels within the normal range has clinical effects and is associated with a number of clinical parameters such as bone mass, BMI, metabolic parameters, and heart rate. The aim of this study was therefore to gain a better insight into the determinants of this between-subject variation in thyroid hormone levels in healthy young men.
Methods
Healthy male siblings ($n=941$, 25–45 years) were recruited in a cross-sectional, population-based study; a history or treatment of thyroid disease and thyroid autoimmunity were exclusion criteria. In these subjects, a complete assessment of thyroid hormone status was performed (TSH, F(T_4), F(T_3), TPO and TG Ab, reverse T_3 (rT_3), TBG and urinary iodine levels). Genotyping was performed by TaqMan SNP Genotyping assays and by KBiosciences.
Results
Total and free T_4, rT_3 and TBG had heritability estimates between 80 and 90%. Calculated estimates for (F)T_3 were considerably lower (60%), while TSH had the lowest estimate (49%). Significant associations were observed between SNPs in the thyroid pathway (rs4704397 in PDE8B, rs10149689 and rs12050077 in TSHR, rs11206244 and rs2235544 in DIO1 and rs13063628 in THRB) and TSH, FT_4, ratio FT_3/FT_4 and rT_3. Nevertheless, these SNPs only explain a limited part of the between-subject variability in thyroid hormones. As to age and life-style related factors, (F)T_3 was negatively related to age and positively to smoking and BMI (all $P<0.0001$) but not to urinary iodine concentrations. Smoking was negatively related to TSH ($P=0.003$) and positively to FT_4 ($P=0.0009$).
Conclusion
Both genetic and life-style related factors play a role in determining between-subject variation in thyroid hormones in euthyroid young men, although genetic factors are most important.

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P978

Efficacy of treatment of cardiac complications in patients with Graves' disease

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Purpose
To assess the dynamics of cardiac complications, the efficacy of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) in patients with thyrotoxicosis
Materials and methods
Prospective 4 years study implicated 340 patients with thyrotoxicosis divided into three age groups: I, 80 patients below 40; II, 132 patients of 40–60; and III, 128 patients above 60. By the beginning of the study atrial fibrillation (AF) was diagnosed in 36% of patients of II group, in 41% – of III group; diastolic

dysfunction – in 19% of I group, in 49% of II, in 50% of III; systolic dysfunction in 36% of II group, 41% of III; left atrial dilatation in 21% of patients of II group, in 30% of III. All patients were treated with Thiamazole (by the 6 month of study were euthyroid), cardioselective beta-blockers. Patients with AF and left auricle dilatation additionally got ACE inhibitors or ARBs. The reference group included 25 patients treated without ACE inhibitors and ARBs.
Results
Diastolic dysfunction remained by the 6th month only in 17% of patients of II group, in 20% – of III; in 24 months – in 16 and 19%, accordingly. Systolic dysfunction remained only in 3% of patients of III group by the 6th month and 1.6% by the year of observation. In patients with ACE inhibitors or ARBs left atrial size and sinus rhythm normalized in 43% in II group and 32% in III group. In reference group only 4% exhibited normalization of the corresponding parametrs.
Conclusion
Cardiac changes in thyrotoxicosis are characterized not only with disturbance of heart rhythm, systolic and diastolic dysfunctions, but also left atrium dilatation. Addition of ACE inhibitors or ARBs permit to decrease left atrium size and to recover sinus rhythm in 43% of middle aged and 32% of senior patients.

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P979

Pilot study of nocturnal TSH surge in idiopathic short stature

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Background
TSH secretion shows a diurnal rhythm with a surge in the evening. The nocturnal surge is essential for thyroid function regulation. TSH surge is suggested to be blunted in central hypothyroidism. In idiopathic short stature (ISS), thyroid dysfunction has been suggested ranging from mild hypothyroidism to central hypothyroidism.
Aim of the work
To study the pituitary thyroid axis and the TSH surge in ISS.
Materials and methods
Thirty subjects (10–18 years old) diagnosed as ISS and thirty matched normal controls were chosen for the study. Thyroid function tests were measured in the basal state, namely T_4,T_3, and TSH. TSH was then measured for each subject at 1000 , 1900, and 2200 h. A peak nadir levels were determined TSH surge (peak-nadir/nadir) was expressed as percent rise over the nadir.
Results and conclusion
33.33% of ISS patients had blunted nocturnal TSH surge (median 10.27) suggesting central hypothyroidism. 33.67% of ISS patients had mild elevation of TSH values and normal nocturnal RSH surge (169.30) suggesting mild hypothyroidism. The remaining 30% of ISS patients had normal thyroid functions and normal nocturnal TSH surge (77.140) as compared to control subjects (60.72) suggesting no role for thyroid dysfunction in this group.
TSH values were lowest at 1900 h in all groups except in mild hypothyroidism at 1000 h. The highest values of TSH were recorded at 2200 h in all groups.
To conclude, the study of TSH surge in ISS is a useful technique to diagnose associated central hypothyroidism and mild hypothyroidism. These patients could benefit from early treatment by levothyroxine.

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P980

Is previous hyperthyroidism associated with long-term cognitive dysfunction? A twin study

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Introduction
Hyperthyroidism has been suggested to adversely affect cognitive function. However, this association could also be caused by genetic and environmental factors affecting both the development of hyperthyroidism and cognitive functioning. By investigating cognitive function within twin pairs discordant for hyperthyroidism, this potential confounding can be minimized. The aim of this study was to examine if hyperthyroidism is associated with long-term cognitive dysfunction.
Methods
Twin pairs discordant for hyperthyroidism were identified by record-linkage between The Danish National Patient Registry, using ICD-8 and ICD-10 codes for hyperthyroidism, and among survey participants of The Danish Twin Registry. Among other investigations, participants had carried out cognitive tests including a Mini-Mental State Exam (MMSE), and six separate cognitive tests. Based on five of the tests a composite cognitive score was calculated. The impact of hyperthyroidism on cognitive function was evaluated by a linear and a conditional logistic regression.
Results
Out of 3036 twin pairs were discordant for hyperthyroidism. The mean time from diagnosis until survey participation was 7.3 years (range: 0–24.1 years). In the inter-pair analysis the hyperthyroid group scored significantly better than the healthy group when controlling for sex, age, zygosity, smoking, and comoborbidities (linear regression: $P=0.038$). In the intra-pair analysis the hyperthyroid twin scored significantly better in the MMSE than did the healthy co-twin (paired t-test; $P=0.023$). When stratifying for time since diagnosis in a paired logistic regression, no statistically significant associations for any of the other cognitive tests were found.
Conclusion
Utilizing discordant twin pairs to control for genetic as well as environmental confounding, we could not demonstrate any clinically relevant negative impact of previous hyperthyroidism on long-term cognitive function.

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P981

The influence of single dose recombinant human thyroid-stimulating hormone on the efficacy of radioiodine therapy in patients with non toxic nodular goitre with low RAIU

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Aim
The aim of our study was to evaluate the influence of recombinant human thyroid-stimulating hormone (rhTSH) on the efficacy of radioiodine therapy in patients with non toxic nodular goitre with low RAIU.
Materials and methods
The study was performed on 50 patients with non toxic nodular goitre (36 females and 14 males, aged 34–75 years) referred for radioiodine therapy. All patients had low RAIU (8–16%), 24 h after a diagnostic dose of I-131 (4 MBq). All the patients received a single intramuscular dose of 0.05 mg rhTSH (thyrogen). 24 h later diagnostic dose of ^{131}I was administered and thyroid scan with RAIU after 24 and 48 h was estimated. Therapeutic dose of I-131 was given on the third day of rhTSH administration. Serum levels of TSH, fT4 and fT3 were determined, 24 and 72 h after rhTSH administration and on the 3rd day after radioiodine therapy. The therapeutic activity of I-131 calculated by Marinelli's formula and ranged between 280 and 600 MBq. The absorbed dose ranged between 150 and 220 Gy. Follow up control was done every 6 weeks. Thyroid ultrasound, and thyroid scan were done again after 6 and 12 months of radioiodine therapy.
Results
A significant increase (2 11 fold) in 24 h RAIU was observed after rhTSH administration. The distribution of radioiodine was more homogeneous 48 h after rhTSH administration. After 12 months 92% of patient were in euthyroid state and 8% develop hypothyroidism. After 6 months the mean reduction in goitre volume was 22 and 45–55% after 12 months. The medium therapeutic activity of I-131 was 280 MBb.
Conclusions
Pre-treatment with rhTSH reduce the therapeutic dose of I-131 by 50–58% without compromising the result of thyroid volume reduction. rhTSH makes radioiodine therapy more effective in the patients with non toxic nodular goitre with low RAIU.

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P982

PRIMARA: a prospective descriptive observational study to review cinacalcet use in patients with primary hyperparathyroidism in clinical practice

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PRIMARA is the first observational study to describe the demographic and clinical profiles of adults with primary hyperparathyroidism (pHPT) receiving cinacalcet in daily clinics across European countries.
Patients with pHPT aged $\geq$18 years without prior cinacalcet treatment were eligible. Initial cinacalcet dosage and subsequent dose changes were at the investigator's discretion. Information on dosing, biochemistry and adverse drug reactions (ADRs) were collected for up to 12 months from enrolment. Analysis was based on observed data.
Of 305 patients enrolled, 219 (72%) completed 12 months of cinacalcet treatment; the main reason for cinacalcet discontinuation was parathyroidectomy (40/86; 47%). 303 patients were evaluable for analysis. Forty-four percent were symptomatic with bone pains (43%) and renal stones (37%) reported as the most common symptoms. Reasons for prescribing cinacalcet included: surgery deemed inappropriate (35%), patient declined surgery (28%) or surgery had previously failed or was contraindicated (22%). Cinacalcet mean (s.d.) starting dose was 43.9 (15.8) mg/day. At month 12, 219 subjects (72%) were still receiving cinacalcet with mean (s.d.) dose of 51.3 (31.8) mg/day.
Median (Q1, Q3) iPTH concentration fell from 16.20 pmol/l (11.14, 27.91) at baseline to 13.85 (8.90, 23.66), 13.95 (8.49, 20.80) and 12.52 (7.97, 21.65) pmol/l at months 3, 6 and 12. At baseline, 22/223 (10%) had albumin-corrected serum calcium of $\leq$2.6 mmol/l; this rose to 63, 69 and 71% at 3, 6 and 12 months respectively. The percentage of subjects achieving reduction in albumin-corrected serum calcium $\geq$0.25 mmol/l was: 56, 63 and 60% at 3, 6 and 12 months post initiation of treatment. ADRs were reported in 81 patients (27%), most commonly nausea.
In conclusion, most patients received cinacalcet because surgery was inappropriate or they declined it. A clinically meaningful calcium decline of $\geq$0.25 mmol/l was observed in 60% of patients, 12 months post cinacalcet initiation without significant safety concerns.

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P983

A comparative study evaluating the roles of inferior thyroid artery blood flow velocities measured by color flow Doppler ultrasonography, TSH receptor antibody and Tc-99m pertechnetate uptake for differential diagnosis between Graves' disease and silent thyroiditis

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Objective
To compare the roles of inferior thyroid artery (ITA) peak systolic and end-diastolic velocities (PSV and EDV) measured by color flow Doppler ultrasonography (CFDUSG), TSH receptor antibody (TRAb) measured by M22 based ELISA and Tc-99m pertechnetate uptake for differential diagnosis between Graves' disease (GD) and silent thyroiditis (ST).
Methods
One hundred and fifty previously untreated subjects with GD (111 female, 39 male, mean age 38.4$\pm$12.8 yeras), 79 with ST (58 female, 21 male, mean age 39.2$\pm$14.1 years) and 71 healthy euthyroid controls (43 female, 28 male, mean age 35.8$\pm$10.5 years)were included in the study. The diagnosis of GD and ST were made according to the patient's signs and symptoms, physical examination findings, the results of TRAb and Tc-99m pertechnetate uptake and follow-up findings. All subjects underwent CFDUSG for the quantitative measurement of ITA blood flow velocities.
Results
The mean ITA-PSV and EDV in patients with GD were significantly higher than those with ST (59.0$\pm$24.6, 25.2$\pm$11.2, 21.4$\pm$5.3 and 9.8$\pm$2.9 cm/s respectively, $P<0.0001$ for both comparisons). However, the mean ITA-PSV and EDV were also significantly higher in subjects with ST than controls (21.4$\pm$5.3, 9.8$\pm$2.9, 17.2$\pm$4.4 and 7.6$\pm$2.3 cm/s respectively, $P<0.0001$ for both comparisons). In ROC analysis the sensitivity/specificity of the 30 and 13.2 cm/s cutoff values of the mean ITA-PSV and EDV for discrimination of GD from ST were 95.3/94.9 and 89.3/88.6% respectively. The sensitivity/specificity of the 1.0 IU/l and 3% cutoff values of the TRAb and Tc-99m pertechnetate uptake were 93.0/91.0 and 90.7/89.9% respectively.
Conclusion
This study suggests that the measurement of ITA-PSV by CFDUSG is a usefull diagnostic tool for differential diagnosis between GD and ST. In ROC analysis the sensitivity and specificity of the most appropriate cutoff value of the ITA-PSV were higher than the sensitivity and specificity of the the most appropriate cutoff values of TRAb and Tc-99m pertechnetate uptake.

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P984

Both Graves' disease and toxic nodular goiter are associated with increased mortality but differ with respect to the cause of death: a Danish population-based register study

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Background
Hyperthyroidism has been associated with increased all-cause mortality. Whether the underlying cause of hyperthyroidism influences this association is unclear. Our hypothesis was that Graves' disease (GD) and toxic nodular goiter (TNG) differ with respect to mortality risk and cause of death.
Methods
An observational cohort study, using record-linkage data from nation-wide Danish health registers. 1.291 and 861 subjects with GD and TNG, respectively, treated in a hospital setting, were identified and followed for a mean period of 11 years. Cases were matched 1:4 with non-hyperthyroid controls with respect to age and sex. The hazard ratio (HR) for mortality was calculated using Cox regression analyses. All analyses were adjusted for co-morbidity, using the Charlson score.
Results
Both GD (HR=1.42, 95% CI 1.25–1.60) and TNG (HR=1.22, 95% CI 1.07–1.40) were associated with increased all-cause mortality. After stratification for the cause of death, GD was associated with increased mortality due to cardiovascular diseases (HR=1.49, 95% CI 1.25–1.77) and lung diseases (HR=1.91, 95% CI 1.37–2.65), whereas TNG was associated with increased cancer mortality (HR=1.36, 95% CI 1.06–1.75). When analyzing mortality in GD using TNG individuals as controls, there was no significant difference in all-cause mortality between GD and TNG. However, GD was clearly associated with a higher cardiovascular mortality (HR=1.39, 95% CI 1.10–1.76) compared to TNG.
Conclusion
Both GD and TNG, treated in a hospital setting, are associated with increased all-cause mortality. The causes of death differ between the two phenotypes with cardiovascular mortality being significantly higher in GD.

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P985

Efficacy and safety of combined parenenteral and oral steroid therapy in Graves' orbitopathy

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Introduction
Glucocorticoids (GC) are the first line treatment for moderate-to-severe and active Graves' orbitopathy (GO), but optimal treatment is still undefined. The aim of the present study was to analyze the efficacy and tolerability of combined parenteral GC pulse therapy followed by oral GC in the interpulse period.
Methods
The study includes 50 patients (48±10 years; 37 females) with untreated, active and moderate-to-severe GO. Patients received 500 mg methylprednisolone in 500 ml physiologic saline followed by oral prednisone tapering dose and repeated each month for the next 5 months. Ophthalmic assessment was performed before and 6 months after treatment. Side effects of GC therapy were evaluated and recorded each month.
Results
GC showed the greatest effectiveness on soft tissue changes (incorporated in the CAS). Median CAS values decreased from 4.5 to 2.0 ($P>0.001$). Thirty-seven patients (74%) demonstrated improvement, there was no change in 13 (26%) patients, and none of the patients presented with deterioration of inflammatory status. Diplopia improved in 21 (42%) patients, was unchanged in 28 (56%) patients, and deteriorated in 1 (2%) patients. There was no significant change in the mean values of proptosis ($P=$NS) at the end of treatment. Improvement in optic neuropathy occurred in 56% of patients. At 6 months, 33/50 patients (66%) demonstrated overall treatment response. Side effects occurred in 35/50 (70%) patients, and vast majority of them were mild and minor. Weight gain and hirsutismus were the most common side effects.
Conclusion
With appropriate selection of patients and careful monitoring during and after treatment, combined parenteral and oral GC therapy is effective and safe.

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P986

Type and extent of morbidity before and after the diagnosis of hypothyroidism: a nationwide register-based study

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Background
Hypothyroidism has been linked with an increased risk of morbidity such as cardiovascular disease and diabetes mellitus. However, it is unknown whether the burden of morbidity is present before or after the diagnosis of hypothyroidism.
Aim
Evaluate the temporality and type of morbidity in hypothyroid patients.
Methods
Observational cohort study. Based on record-linking between official Danish health registers 2822 subjects with hypothyroidism were identified and matched with four non-hypothyroid controls and followed over a mean period of 6 years. Prevalence of cardiovascular diseases (CVD), lung diseases, diabetes mellitus (DM), rheumatic diseases (RHD) and malignant diseases were recorded. Logistic and cox regression models were used to assess the risk of morbidity before and after the diagnosis of hyperthyroidism, respectively.
Results
In subjects with hypothyroidism, the odds ratio (OR) was significantly increased for CVD (OR 1.37; 95% CI: 1.19–1.58), lung diseases (OR 1.25; 95% CI: 1.13–1.39), DM (OR 1.92; 95% CI: 1.61–2.29) as well as malignant diseases (OR 1.24; 95% CI: 1.06–1.45), prior to the diagnosis of hypothyroidism. The hazard ratio (HR) for disease after the diagnosis of hypothyroidism was significantly increased for CVD (HR 1.36; 95% CI: 1.15–1.60); lung diseases (HR 1.51; 95% CI: 1.30–1.75); and DM (HR 1.40; 95% CI: 1.11–1.77).
Conclusions
Prior to the diagnosis of hypothyroidism there is an increased risk of being diagnosed with CVD, lung diseases, DM, and malignant diseases. Following the diagnosis of hypothyroidism there is an increased frequency of CVD, lung diseases, and DM.

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P987

Influence of thyrotropin on human peripheral blood immune cell populations

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Introduction
Dendritic cells (DCs) are considered as main regulators of immune system. Functional properties of DCs depend on their subtype, maturation status, interaction with other immune cells as well as environmental factors including hormones. Recently, we have shown a direct, independent of TSH, regulatory influence of thyroid hormones on human DCs function. The aim of the present study was to analyze *ex vivo* the effect of systemically administered TSH on human peripheral blood immmune cell populations, potentially interacting with DCs and modulating their function.
Methods
Blood samples for the flow cytometry analysis of peripheral blood plasmacytoid and myeloid DC subtypes were collected from patients thyroidectomized because of differentiated thyroid cancer at two consecutive time points: i) directly before the commencement of TSH administration and ii) 5 days after first TSH injection. The whole blood quantitative and phenotypic analysis of immune cell populations was performed by flow cytometry.
Results
As previously shown, systemic administration of TSH did not influence the percentage and maturation status of plasmacytoid and myeloid DCs in peripheral blood of thyroidectomized patients. Interestingly, we observed a significant increase of CD16 positive PBMCs. This difference was dependent specifically on natural killer (NK) cells whereas the CD16+ monocyte fraction did not change after TSH administration.
Conclusions
In the present study, the systemic TSH administration resulted in the specific increase of CD16+ NK cells fraction in peripheral blood of thyroidectomized patients. These results are of great importance for the understanding of endocrine-immune regulatory network in humans.

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P988

Hemithyroidectomy for benign euthyroid goiter increases the mitochondrial membrane potential of peripheral mononuclear blood cells

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Introduction
Patients who do not develop overt hypothyroidism after hemithyroidectomy for benign euthyroid goiter have permanently increased serum levels of TSH and decreased levels of thyroid hormones within the reference ranges. Thyroid hormones are major regulators of mitochondrial function, and the mitochondrial membrane potential (MMP) can be measured by flow cytometry analysis of living cells as the fluorescence intensity of stained peripheral mononuclear blood cells (MNBCs). We have previously shown increased MMP of MNBCs in patients with subclinical hypothyroidism. Increased MMP might represent increased production of reactive oxygen species rather than of ATP.
Aim
To determine if the hemithyroidectomy-induced change in TSH and thyroid hormones affects the mitochondria of peripheral MNBCs.
Method
In an ongoing prospective study, patients are examined at one time point before and at four time points (1, 3, 6, and 12 months) after hemithyroidectomy for benign euthyroid goiter. TSH, fT4 and tT3 are measured and the MMP is measured as the fluorescence intensity of MitoTracker Green (MTG)- and Tetramethylrhodamine methyl ester (TMRM)-stained MNBCs by flow cytometry analysis.
Results
We present a 6-month follow-up of 22 hemithyroidectomized patients who do not receive levothyroxine treatment. TSH shows a persistent increase (median

0.97 mUl/l versus median 2.95 mUl/l, $P<0.000$), and fT4 (median 16.0 pmol/l vs median 15.20 pmol/l, $P<0.003$) and tT3 a persistent decrease (median 1.89 nmol/l vs median 1.73 nmol/l, $P<0.004$) within the reference ranges, unchanged to values 1 month after hemithyroidectomy. The MMP of MNBCs was persistently increased after six months (median 4079 TMRM fluorescence a.u. vs median 6327 a.u., $P=0.002$).

Conclusion

Although they are considered clinically and biochemically euthyroid, hemithyroidectomized patients have lowered thyroid function and hyperpolarized mitochondria. It is unknown if these effects of hemithyroidectomy have clinical consequences.

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P989

Influence of smoking on hyperthyroidism severity in newly diagnosed Graves' disease patients

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Introduction

Smoking is an independent risk factor for Graves' disease (GD) and an independent predictor for Graves'ophtalmopathy (GO), but the influence on hyperthyroidism severity was not established.

Aim

To evaluate the relationship between smoking and thyroid function at diagnosis and after administration of antithyroid drugs (ATDs) in GD patients.

Materials and methods

We prospectively investigated 116 newly diagnosed, untreated GD patients, 82.8% females, mean age 43.8 ± 15.2 years. At diagnosis we recorded: demografic characteristics, smoking status (non-smoker 63.8%, current smoker 36.2%), presence of ophtalmopathy, goiter size, autoantibody titer, serum concentrations of TSH, FT_4, FT_3, TT_3, and usual biochemistry. All patients received treatment with methimazole and were followed (mean 12 ± 9 months) at 2 months with physical exam and FT_4, TT_3, TSH at least until TSH levels returned to normal (>0.4 mUI/ml). We recorded the time needed for the return of thyroid hormones (THRN) and TSH (TTRN) levels to normal.

Results

There was no difference in current smoking percentage between females (33.3%) and males (50%), $P=0.15$. Smoker males presented lower FT_3 ($P<0.001$), higher FT_4/FT_3 ($P<0.001$) at diagnosis and longer THRN ($P<0.001$) when compared to non-smoker males. Age at ophtalmopathy onset was significantly lower in current smokers (34.3 ± 11.7 vs 47.9 ± 13.8, $P=0.008$), but we found similar percentages of ophtalmopathy between smokers and non-smokers. Smokers >40 years presented higher FT_4 ($P=0.049$), TT_3 ($P=0.032$) and FT_4/FT_3 ($P=0.034$) at diagnosis compared to non-smokers >40. Compared to nonsmokers, current smokers associated higher prevalence of medium/ large goiters (78.6 vs 62.2%, $P=0.025$). We found no difference between TSH, FT_4, TT_3, FT_3, antibody titer at diagnosis, the THRN and TTRN between current smokers and non-smokers.

Conclusions

Current smoking is associated with younger age at ophtalmopathy onset, larger goiters, and, generally, does not seem to influence hyperthyroidism severity at diagnosis or the time needed until thyroid hormones and TSH levels return to normal.

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P990

Postprandial studies uncover differing effects on HDL particles of overt and subclinical hypothyroidism

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Overt hypothyroidism (OH) and more controversially subclinical hypothyroidism (SCH) are associated with abnormal lipid metabolism and endothelial dysfunction under fasting conditions. Little data exists regarding the metabolic and vascular effects of OH or SCH under postprandial conditions.

We aimed to characterize postprandial metabolism with emphasis on intestinally derived lipoproteins, HDL-cholesterol (HDL-C) and endothelial function in patients with OH, SCH and normal subjects.

Subjects were studied fasting and for 8-h following a mixed-meal. Apolipoprotein (Apo) B48, a marker of intestinally-derived lipoproteins was measured by ELISA. Systemic and HDL-associated inflammation was assessed by measuring serum-amyloid-A (SAA)-levels. HDL was subfractionated into HDL2 and 3 by rapid ultracentrifugation. Cholesteryl-ester-transfer-protein (CETP), which mediates transfer of cholesterol from HDL to triglyceride-rich lipoproteins and LDL-cholesterol (LDL-C) was measured in HDL2 and 3 subfractions. Flow-mediated-dilatation (FMD) of the brachial artery was measured to assess endothelial dysfunction.

Compared to normal subjects, postprandial ApoB48 AUC was greater in OH and SCH while postprandial HDL-C was lower in SCH but not OH. There were no significant between-group differences in LDL-C, triglycerides, or SAA. HDL2- and 3-associated CETP activity was lower in OH compared to normal and SCH subjects. FMD was reduced in OH compared to SCH and normal subjects postprandially.

Table 1 Median values.

	ApoB48 (mmol/l)	HDL-C (mmol/l)	HDL2-associated CETP (μg/mg)	HDL3-associated CETP (μg/mg)	FMD% change
Controls Fasting ($n=42$)	7.5	1.4	2.2	316.4	5.9
SCH Fasting ($n=21$)	11.0	1.2	1.9	291.2	6.0
OH Fasting ($n=21$)	12.5#	1.4	1.8*	269.9*	4.4
Controls Postprandial	12.4	1.3	2.0	328.2	6.0
SCH Postprandial	18.6*	1.1*	1.7	299.0	5.7
OH Postprandial	26.0 #	1.3	1.6*	289.5*	3.6*

*$P<0.05$ vs.controls and #$P<0.001$ vs controls.

Postprandial lipoprotein and vascular abnormalities differ between OH and SCH. Although both are characterized by increased intestinally-derived lipoprotein particles, HDL-C is reduced only in SCH. Maintained HDL-C in OH probably reflects reduced CETP, which was not observed in SCH. Postprandial endothelial dysfunction is abnormal only in OH and does not appear to reflect increased inflammation.

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P991

Quantification of tissue T_3 and T_4 in rat and human heart by a novel HPLC-MS/MS method

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Thyroid hormone (T_3) is mostly produced in peripheral tissues by thyroxine (T_4) deiodination, and its functional effects are directly related to tissue concentration, which is quite difficult to assay by conventional immunological methods. We report a novel technique, based on tandem mass spectrometry (MS/MS) coupled to HPLC, which was used in left ventricular myocardium obtained from rat and human hearts.

Samples were homogenized in phosphate buffer (pH 7.4). After centrifugation, supernatants were spiked with stable-isotope-labelled internal standards (IS: $^{13}C_6$-T_3 and $^{13}C_6$-T_4) and extracted by SPE. Dried residues were reconstituted and incubated with 3.0 N HCl in n-butanol, obtaining the butyl esters of T_3, T_4, and ISs. After removing excess reagents, residues were reconstituted with methanol/HCl 0.1 M (50:50 v:v) and injected in the HPLC-MS/MS system (AB-Sciex API 4000). Calibration curves were built with standard solutions containing both analytes at concentrations ranging from 1 to 50 ng/ml and the same amount of IS as put in the samples.

Sample derivatization increased method sensitivity and accuracy, and the minimum amount of tissue needed was ~100–150 mg. In control rat myocardium, tissue T_3 and T_4 averaged 1.59 ± 0.11 and 2.24 ± 0.22 pmol/g respectively (corresponding plasma free T_3 and T_4 were 3.69 ± 0.39 and 17.09 ± 1.56 pM). In animals treated with low-dose (6 μg/kg per day) or high-dose (45 μg/kg per day) T_3, tissue T_3 increased to 3.12 ± 0.29 and 6.76 ± 1.37 pmol/g

(plasma free T_3 was 8.06±0.83 and 19.59±3.80 pM), while tissue T_4 decreased to 0.79±0.06 and 0.77±0.02 pmol/g (plasma free T_4 was 6.34±1.41 and 3.49 ±0.43 pM). In human samples obtained from transplanted hearts T_3 and T_4 averaged 1.51±0.16 and 5.94±0.63 pmol/g.
In conclusion an HPLC-MS/MS method based on derivatisation with buthanol enables T_3 and T_4 assay in ≥150 mg myocardial samples. Tissue T_3 and T_4 assay may be critical to understand the role of thyroid hormones in physiological and pathophysiological conditions.

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P992

Study on linear growth and thyroid function for 12 years in patients with β thalassemia major

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Introduction
Short stature and hypothyroidism and are frequent complications of thalassemia major (TM).
Patients and methods
We recorder growth parameters and investigated the thyroid function in TM patients attending our Pediatric Endocrine Clinics for 12 years. Definitions: overt hypothyroidism (low FT_4 and increased TSH levels >5 μIU/ml); subclinical hypothyroidism (normal FT_4, TSH between 5–10 μIU/ml) and central (secondary) hypothyroidism (low FT_4 and normal or decreased TSH).
Results
48 patients completed a 12 years of follow-up. Hypothyroidism was diagnosed in 35% of patients without gender difference. There was a progressive decrease of height SDS (from −0.2±0.3 to −1.78±0.5) and BMI SDS (from 0.2±0.3 to −0.6±0.35). HtSDS was <−2 in 46% of patients. The general trend of free thyroxine level showed progressive decrease over the 12 years, whereas TSH levels did not show a corresponding increase. 94% patients had hypothyroidism after the age of 10 years. Overt hypothyroidism had risen from 0% at the age of 7 years to 35% at the age of 18 years. None had high anti-thyroperoxidase (TPO) antibody titers. 13/17 patients with hypothyroidism, had normal or low TSH level (not appropriately elevated) indicative of defective hypothalamic pituitary response to low FT_4 (central hypothyroidism). 3/17 patients had subclinical hypothyroidism (TSH between 5 and 10 μIU/ml and normal FT_4). There was a significant negative correlation between serum ferritin and FT_4 ($r=-0.39$, $P=0.007$) and between FT$ and HTSDS ($r=0.52$, $P<0.001$).
Conclusions
Progressive height loss and worsening of thyroid function was observed in 46 and 35% respectively in thalassemic patients by the age of 18 years. The lack of proper increase of TSH in response to low levels of FT_4 in (76%) of these patients indicated a high incidence of defective pituitary thyrotrophic function in these patients.

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P993

Levothyroxine requirements in thyroidectomized diabetic patients receiving metformin

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Background
Recent studies suggest that metformin (MF) may reduce TSH concentration. This fact could imply a dosage reduction of levothyroxine among those hypothyroid patients taking metformin.
Aim
To determine, in a retrospective analysis, the impact of metformin in thyroidectomized patients (a condition not influenced by endogenous thyroid hormone production) on levothyroxine replacement.
Patients and methods
One hundred ninety two subjects underwent total thyroidectomy. Patients were divided into two groups depending on MF use: group A, without metformin (159 patients: 134 women; 52 (15.7) years old, 70.2 (13.5) kg of weight, 56 of them affected with differentiated thyroid cancer (DTC)), and group B, with metformin (33 patients: 24 women; 63 (9.8) years old, 79.3 (13.9) kg of weight, nine cases with DTC). Replacement dose was properly adjusted in all patients. Levothyroxin requirements were compared between groups.
Results
TSH levels did not show statistically significant differences between the groups: TSH 0.67 (0.11–2.81) mU/l in group B and 0.80 (0.11–4.28) mU/l in group A; P value=0.46. No differences on total levothyroxine dosage were found: 114 (100–150) μg in group A vs 125 (100– 142) in group B; P value=0.9. However, when calculating the weight adjusted levothyroxine dose (μg/kg) significant differences were evidenced: 1.66 (1.38–2.08) μg/kg in group A vs 1.53 (1.26–1.70) in group B; P value=0.010.
Conclusions
Thyroidectomized patients on metformin treatment do need lower thyroxine dose per kilogram.

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P994

Association between the use of computed tomography scans using iodine-based contrast medium and the development of subsequent hyperthyroidism

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Background and aim
There is an increasing use of computed tomography (CT) scans with an iodine-based contrast medium. Last year, a large University Hospital in Denmark performed 50 000 CT scans where each injection contained between 3500 and 7000 μg free iodine corresponding to 23–45 times normal daily intake. We wished to investigate if patients with newly diagnosed hyperthyroidism had been exposed to an iodine based CT scan within 1 year prior to symptoms of hyperthyroidism.
Materials and methods
All patients with newly diagnosed hyperthyroidism (either first time episode or recurrent) through 1 year (2010) were linked individually with a register for CT scans performed in 2009 and 2010. Only cases where a CT scan was performed prior to an outbreak of hyperthyroidism were included.
Results
230 new cases of hyperthyroidism were originally classified as: 101 patients with Graves' disease (73 new, 28 with recurrent), 72 with multinodular goitre, 9 with amiodarone or iodine-induced hyperthyroidism, 8 with HCG-induced hyperthyroidism, 26 with subacute thyroiditis, 11 with postpartum thyroiditis, 1 with painless thyroiditis, 1 was Ipilimumab-induced, and finally 1 induced by Interferon. Fourteen patients, corresponding to 0.0003% of all contrast injections, but 6.1% of all patients with hyperthyroidism, had undergone a CT scan before the outbreak of hyperthyroidism. Based on type of hyperthyroidism, 1/101 (1%) had Graves' disease, 11/72 (15%) had multinodular goitre and the last two had hyperthyrodism induced by Ipilimumab and Interferon.
Conclusion
From a radiologist's point of view: a CT scan with iodine-based contrast induces extremely seldom hyperthyroidism. From an endocrinologist's point of view: a CT scan with iodine-based contrast performed within 1 year prior to symptoms seems significantly associated with outbreak of hyperthyroidism in a multinodular gland (15%) as opposed to Graves' disease.

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P995

Metformin decreases thyroid volume and nodule size in subjects with insulin resistance

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Introduction
Previous reports have shown that subjects with insulin resistance have increased thyroid volume and nodule prevalence. We investigated the effects of metformin on thyroid volume and nodule size.
Methods
This prospective data were gathered on newly diagnosed subjects with insulin resistance ($n=100$, 68 female, 32 male) between August 2008 and May 2010.

Measurements of thyroid hormones and thyroid ultrasonography was performed in all participants before and after 6 months therapy of metformin.
Results
Mean BMI and waist circumference decreased significantly after metformin therapy (34.5±5.1 vs 32.7±4.8 kg/m^2 and 106.3±11.8 vs 101.8±19.0 cm respectively) ($P<0.0001$ for BMI; $P=0.008$ for waist circumference). Insulin resistance estimated by homeostasis model assessment also decreased after metformin therapy (4.5±1.9 vs 2.9±1.7, $P<0.0001$). After metformin therapy, mean TSH level was lower (1.8±1.0 vs 1.5±0.8 mIU/l, $P<0.0001$), mean free T_3 was higher (2.7±0.7 vs 3.0±0.8 pg/ml, $P=0.03$), and mean free T_4 was similar (1.4±0.6 vs 1.5±1.3 ng/dl, $P>0.5$). Mean thyroid volume (22.5±11.2 vs 20.3±10.4 ml, $P<0.0001$) and mean thyroid nodule size (12.9±7.6 vs 11.7±7.2 mm, $P<0.0001$) also decreased after treatment.
Conclusions
In subjects with insulin resistance, metformin therapy significantly decreases thyroid volume and nodule size, which seems to not only shed light on novel aspects in pathophysiology of goiter, but also to introduce therapeutic challenges as well.
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P996

A case of severe hypoglycaemia in a patient with hyperthyroidism
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Introduction
Graves' disease is a common condition which is usually treated with antithyroid drugs like carbimazole and propylthiouracil. Here we present a patient who developed severe hypoglycaemia after treatment with carbimazole for hyperthyroidism which is unusual in Indian population.
Case report
A 31-year-old male Indian presented to Endocrine Clinic with typical signs and symptoms of thyrotoxicosis. His biochemistry showed FT_3: 28.16 pg/ml (1.8–4.6), FT_4: 20.5 ng/dl (0.93–1.7), TSH: 0.005 μIU/ml (0.27–4.2) and random blood sugar was 90 mg/dl. Technetium thyroid scan showed high uptake of 13.4% and Graves's disease was confirmed, following which he was treated with carbimazole and propranolol. Two weeks later the patient presented to hospital with unconsciousness, frothy discharge from mouth, seizures and sweating. Severe hypoglycaemia confirmed with blood sugar of 29 mg/dl. In the hospital he had repeated episodes of hypoglcyaemia which was treated symptomatically. Further investigation revealed normal cortisol level but his inulin level was elevated at >1000 pmol/l, when his blood sugar was 43 mg/dl. His antiinsulin antibody was also elevated at >300 U/ml (positive >18). He was found to have insulin autoimmune syndrome (IAS, Hirata's Disease) induced by antithyroid medication carbimazole. He was taken off the carbimazole and treated with radioactive iodine therapy. Within a few months he was rendered asymptomatic.
Conclusion
Insulin autoimmune syndrome is a quite rare condition and associated with a strong genetic predisposition. The first case was reported in 1970 by Hirata and majority was reported from Japan and it has not been reported in Indian population. Underlying autoimmune disorders or exposure to specific drugs were presumed to be responsible for the development of insulin autoimmune syndrome especially sulphydril drugs such as methimazole, alpha mercaptopropionyl glycine, and gluthatione, hydralazine, isoniazide, procainamide, and penicillin. It is important to recognize the condition at the earliest and treat appropriately.
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P997

Associations between urinary phthalate metabolites and thyroid function: pilot study
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Background
Phthalates presents widespread endocrine disrupting chemicals in many personal-care and consumer products. A limited number of experimental and human studies suggest that exposure to phthalate may be associated with altered thyroid function, but a problem is not elucidated enough. This study focused to examination of the association between urine phthalate metabolites and thyroid function.
Methods
We included 91 healthy person, 47 females and 44 males, mean age 35.91±8.10 years. According to presence (group A) or absence (group B) of urinary phthalate metabolites participants divided in two groups. Free thyroxin (FT_4), free triiodothyronine (FT_3) and thyroid-stimulating hormone (TSH) were measured. Phthalate monoester metabolites (MEP-monoethyl phthalate, MBP-monobuthyl phthalate, MOP-monooctylphthalate, MEHP-mono-(2-ethylhexyl)-phthalate) were measured in single spot urine by mass spectrometry.
Results
In all group values of thyroid hormones and TSH were in normal range (FT_4 13.41±1.49 pmol/l, FT_3 5.00±0.64 pmol/l, TSH 1.87±0.98 mIU/l). Urine phthalate metabolite values were: MEP 36.40±137.46; MBP 22.94±118.34; MOP 16.43±102.01; MEHP 44.55±83.30 ng/ml. Significant negative correlation were between FT_4 and MEP ($r=-0.214$; $P<0.05$) and significant positive between TSH and MEP ($r=0.444$; $P<0.01$). In group A values of FT_4 and FT_3 were slightly decreased and TSH increased than in group B. Urine values of phthalate metabolites in group A were: MEP 71.21±186.65; MBP 22.94±118.34; MOP 16.43±102.01; MEHP 44.55±83.30 ng/ml. Significant negative correlation were found between FT_3 and MEP ($r=-0.300$; $P<0.05$) and borderline between FT_4 and MEP ($r=-0.282$; $P=0.06$). Statically significant positive correlation was between TSH and MEP ($r=0.542$; $P<0.01$).
Conclusion
Exposure to DEP (diethylphthalate) may affect thyroid function. Further studies are needed to elucidate this association.
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P998

Selective embolization of thyroid arteries (SETA) in patients with Graves' disease
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Introduction
Prevention of surgical complications in patients with severe forms of Graves' disease is still not completely solved problem. The purpose of research was improve the results of surgical treatment of patients with severe Graves' disease.
Materials and methods
In 2012 introduced a method of treatment of patients with Graves' disease SETA as a preoperative preparation. Presented results of surgical treatment of 30 patients, with severe forms of Graves' disease. The patients were divided into two groups. Main group consisted of 15 patients before strumectomy using SETA, was 5 (33%) men and 10 (67%) women aged 26–52 years old,and in the control group, in which 15 patient performs subtotal strumectomy, was 4 (26.6%) men and 11 (73.3%) women aged 26–63 years.
Results
SETA way used by us as pre resective preparation for 3–4 days prior to strumectomy. During this time, achieved by reducing the volume of the thyroid, the reduction of blood flow in the thyroid tissue, potentiation thyrostatic preoperative therapy, a significant reduction in intraoperative blood loss. The degree of thyroid enlargement in all patients corresponded to grade two (by WHO), with decompensated thyrotoxicosis. To all 30 patients performed subtotal strumectomy. The total duration of the operation in the control group was on average 63.7±6.1 min. In the basic mean duration of surgery was less and was 45.4±5.8 min. In the analysis of postoperative complications in 1 (6.3%) patients of the control group was thyrotoxic crisis, moderate postoperative bleeding was observed in 3 (20%) in the control group. In the basic group without complications. No deaths among patients.
Conclusions
SETA in patients with severe Graves' disease in the pre resective period in considerable reduction effects of hyperthyroidism, thyroid volume reduction compared to the original, enables significantly reduced intraoperative blood loss, which facilitates the technique of the operation. SETA can reduce operating time to 26%, the amount of intraoperative blood loss – to 52.5%, and avoid bleeding in the early postoperative period. Further study results SETA also as an independent treatment option for Graves' disease.
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P999

Short-term effects of thyroid hormone therapy on quality-of-life evaluation by ThyPRO questionnaire in chronic autoimmune thyroiditis

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In chronic autoimmune thyroiditis, increased TPOAb levels have been found to be associated with impaired quality-of-life (QoL). Aim of the study was to evaluate QoL by means of ThyPRO in women with positive TPOAb levels. From January to December 2011, 62 women were invited to the study. Inclusion criteria were: age >18 years, TPOAb >100 mIU/l, no previous therapy for thyroid, no current wish to become pregnant and availability of an E-mail address. Women were randomized to observation (Gr 1 $n=21$), L-T_4 (Gr 2 $n=21$; 50 μg/day) or L-T_4 + T_3 (Gr 3 $n=20$; 37 μg + 10.7 μg/day). Clinical parameters, symptoms, current therapies and laboratory parameters were recorded. Women were asked to compile and send in ThyPRO at the baseline and 1 and 6 months after. No differences among groups noted with regard to age and clinical and laboratory parameters. At the baseline TSH >4.2 mIU/l was found in 62, 81, and 80% in Gr 1, Gr 2, and Gr 3. No differences in symptoms or drug load were noted at the baseline, but asthenia was more frequent in Gr 3 (45%) than in Gr 1 (24%) and Gr 2 (9%) ($P=0.03$). No difference in psychological symptoms (Gr 1 19%, Gr 2 33%, Gr 3 40%; $P=0.3$) or therapies (Gr 1–3 1–2%) was found. The study was completed by 71, 57, and 60% of Gr 1, Gr 2, and Gr 3 women. At the baseline, no difference in ThyPRO scores emerged. Only systolic BP was significantly ($P=0.02$) related to the mean ThyPRO score. A decrease in TSH levels was noted in Gr 2 ($P=0.001$) and Gr 3 ($P=0.01$). QoL improved but, the decrease in mean ThyPRO scores (i.e. fewer symptoms or lower impact of disease) was only slightly significant in Gr 1 (18.1 ± 1.8 vs 15.7 ± 2.2; $P=0.02$), while it was highly significant ($P=0.0005$) in Gr 2 (30.1 ± 4.9 vs 20.3 ± 3.9) and Gr 3 (26.1 ± 3.5 vs 16.7 ± 2.6). At the last examination, a significant difference was found in % of symptomatic patients among the groups (Gr 1 33%, Gr 2 42%, Gr 3 83%; $P=0.02$). At the baseline, the scale Tiredness displayed the highest scores; at the last examination, an improvement was noted, but this was significant only in Gr 3 ($P=0.03$). Although further studies in larger populations are needed, our data do not confirm a correlation between QoL and TPOAb levels in autoimmune thyroiditis. A significant improvement in QoL was seen and this was more significant under thyroid hormone supplementation. The differences between L-T_4 therapy and L-T_4 + T_3 therapy proved slight.

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P1000

Genetic examination of the TSHR gene in patients with congenital hypothyroidism: systematic survey of a Hungarian cohort

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Loss-of-function mutations in the TSH receptor (TSHR) gene are one of the most common known causes of congenital hypothyroidism (CH). While heterozygous mutations result in nonautoimmune isolated hyperthyrotropinemia, homozygous and compound heterozygous mutations may cause overt CH of various severity depending on the localization and type of the mutations.
In our study we performed the systematic genetic analysis of the TSHR gene of a cohort of 85 Hungarian patients diagnosed postnatally with CH in Szeged, one of the two Hungarian centres involved in the neonatal TSH screening program. Patients' detailed clinical data were collected and DNA was isolated from peripheral blood. Genetic analysis was implemented at the Division of Endocrinology and Metabolic Disease, Laboratory of Endocrine and Metabolic Research, Italian Auxological Institute, as follows. Exons and their immediate flanking intronic sequences were PCR amplified. Examination of the resulting PCR fragments were performed either with denaturing HPLC (DHPLC), or with direct sequencing, where it was appropriate. In those cases, where DHPLC result indicated genetic alteration, sequencing was also performed.
As a result, beside polymorphic variants, we identified six missense mutations in four patients, among which two mutations are new, so far unidentified naturally occurring mutations (N432D and P449L). Patients 24 and 79 harboured heterozygous mutations (N432D and P162A respectively), whereas Patients 52 and 58 were compound heterozygotes (P162A–P449L and C41S–P162A respectively).
As all four patients had overt CH, genetic examination of the post-transcriptional regulatory elements of the TSHR gene in the heterozygous patients may be also considered. These results along with future genetic examination of the patients' families, as well as the *in vitro* functional studies of the new mutations may help us deciphering further details of the complex signalling mechanism through TSHR.

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P1001

In pregnant women receiving levothyroxine, higher 3rd trimester TSH and maternal underweight are associated with cesarean section due to foetal malposition

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Context
In the background population, maternal TSH levels >2.5 mUI/l in late pregnancy have been reported to be associated with breech presentation. This has not been addressed in women treated with levothyroxine.
Aim
Our aim was to study the relationship of 3rd trimester maternal TSH with caesarean section (CS) due to foetal malposition in pregnant women treated with levothyroxine since before pregnancy.
Methods
We have studied 222 women with primary pregestational hypothyroidism or differentiated thyroid carcinoma treated with levothyroxine since before pregnancy and delivering at a gestational age of 22 weeks or more. Women with pregestational diabetes mellitus and multiple pregnancies were excluded. As potential predictors of CS due to malposition we have considered 3rd maternal TSH, maternal age, BMI classification, nulliparity, gestational age at delivery, foetal sex, birth weight, small/adequate/large weight for gestational age, and major malformations. Statistical analyses: data are expressed as percentage or median (P25, P75), analyses include a logistic regression analysis with CS due to foetal malposition as the dependent variable and using all the previously mentioned potential predictors.
Results
Maternal characteristics were: age 33 (30–36) years, prepregnancy BMI 23.33 (21.4–23.8) kg/m^2, mean 3rd trimester TSH 1.5 (0.3–2.6). The most frequent underlying diseases were hypothyroidism (50%), hypothyroidism after treatment for Graves' disease (27.6%) and post-surgery differentiated thyroid carcinoma (15.3%). The rate of CS due to foetal malposition was 2.7%. Logistic regression analysis to predict CS due to foetal malposition identified 3rd trimester maternal TSH and maternal underweight as potential predictors, with odds ratios of 1.29 (1.1–1.5) and 13.9 (1.17–165.5) respectively.
Conclusions
In women receiving pregestational treatment with levothyroxine, maternal 3rd trimester TSH and underweight are predictors of CS due to foetal malposition. This extends the findings in the general obstetric population.

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P1002

Diagnosis of hypothyroidism is associated to an increased risk of acute decompensated heart failure occurrence, but not of mortality among heart failure outpatients

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It has been demonstrated that hypothryoidism could affect the prognosis of patients with cardiovascular disease. The aim of this study was to better clarify the prognostic role of hypothyroidism in patients with chronic heart failure (CHF). We enrolled 384 consecutive outpatients (65 ± 13 years, 296 males, NYHA 2.3 ± 0.6, left ventricular ejection fraction, LVEF, 32 ± 9%) with CHF (ESC criteria), in stable clinical conditions (at least 30 days) and in conventional

therapy (at least 6 months) (91% ACE-inhibitors and/or AT1R antagonists, 89% betablockers, 87% diuretics, 54% aldosterone antagonists). The presence of hypothyroidism was defined according to a previous diagnosis of hypothyroidism or to its diagnosis at the enrolment or during follow-up. Overt hypothyroid patients and patients with subclinical hypothyroidism and a TSH $\geq$10 mU/l were always treated with levothyroxine, aiming to have a TSH in the normal reference range. Patients with a TSH slightly above the normal range but $<$10 mU/l were either treated or simply revaluated over the time. Patients with hyperthyroidism were excluded.

In 91 (24%) patients of study population hypothyroidism was detected. During follow-up (31$\pm$10 months), a total of 98 patients were hospidalized for acute decompensated heart failure (ADHF) and 58 died. A diagnosis of hypothyroidism was significantly associated to ADHF occurrence at univariate (HR: 2.17; 95% CI: 1.44–3.27; $P<0.001$) as well as at multivariate Cox regression analysis (HR: 1.58; 95% CI: 1.02–2.45) after correcting for age, presence of ischemic cardiomyopathy, arterial systolic pressure, NYHA class, LVEF and levels of creatinine, Sodium and NT-proBNP. No association was found, however, between diagnosis of hypothyroidism and mortality (HR: 1.15; 95% CI: 0.639–2.07; *P*: NS). Fig. shows Kaplan Meier curves for events in patients with and without a diagnosis of hypothyroidism.

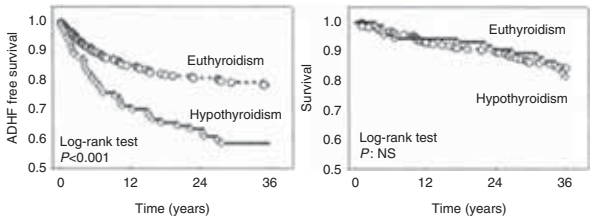

Figure 1

In conclusion, in CHF outpatients the diagnosis of hypothyroidism is independently associated to a greater probability of ADHF occurrence but not of mortality.

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P1003

Thyroid-stimulating hormone and insulin resistance indexes in women with polycystic ovary syndrome

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Thyroid dysfunction has been linked to reduced fertility. It is known that changes in SHBG and sex steroids are a consistent feature associated with hyper- and hypothyroidism. It is also has reported that women with polycystic ovary syndrome (PCOS) are more insulin-resistant than it would be expected on the basis of their age and BMI. The mechanisms underlying this phenomenon are still not clarified. Hyperandrogenaemia may play a role, but also other factors such as thyroid function may also be involved. The aim of this study was to estimate the interrelationship between thyroid-stimulating hormone (TSH) levels and insulin resistance (IR) indices in women with PCOS. We included in the study 55 women (age: 32.18$\pm$0.63 years; BMI: 25.34$\pm$0.59 kg/m^2) diagnosed as polycystic ovary syndrome (PCOS) according Rotterdam criteria. Thyroid-stimulating hormone (TSH), oral glucose tolerance testing (OGTT), homeostatic model assessment of insulin resistance (HOMA-IR), androgen levels (total testosterone and androstenedione), free androgen index (FAI) and sex hormone-binding globulin (SHBG) were performed. All women had normal glucose tolerance during OGTT. All women had normal TSH level (conventionally used levels of 4–5 mIU/l as upper limit). Using nonparametric Spearman's correlation it is showed significant correlation ($P<0.05$) among TSH level and investigated parameters: SHBG ($-$0.317), HOMA-IR (0.358); fasting insulin (0.455), insulin at 120 min of OGTT (0.369), FAI (0.466) and androstenedione (0.407) respectively). All correlation was repeated after adjusting for BMI and age and these significant correlations were confirmed. Our data suggested that in patients with PCOS, a significant association between level of TSH and insulin resistance indexes as well as SHBG and androgen levels exist, and this association appeared to be independent of age and BMI.

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P1004

Overweight/obese women with primary acquired hypothyroidism in appropriate levothyroxine replacement therapy are characterized by impaired whole body energy metabolism

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Introduction

Appropriately titrated levothyroxine (L-T_4) replacement therapy may not be able to fully correct the entire set of metabolic defects afflicting individuals with primary hypothyroidism. Based on this hypothesis, the present study was undertaken to establish whether these patients have impaired whole body energy metabolism.

Methods

We recruited 30 hypothyroid women with duration of the disease $>$2 years, BMI $>$25 kg/m^2 and serum TSH $<$3.5 μU/ml under replacement therapy with L-T_4 (mean dose: 73$\pm$34 μg/die) and compared them to 18 eu-thyroid women matched for age (53$\pm$13 vs 48$\pm$10 years), BMI (32.5$\pm$7.0 vs 33.7$\pm$8.3 kg/m^2), menopausal state and life-style habits ($P>0.3$ for all). They underwent bioelectrical-impedentiometry (BIA) and indirect calorimetry to assess body composition and resting energy expenditure (REE) respectively.

Results

TSH (1.92$\pm$1.06 vs 1.87$\pm$0.89 μU/ml; $P=0.91$) and body composition (body fat: 41.4$\pm$7.4 vs 42.1$\pm$8.3%; LBM 58.6$\pm$7.4 vs 57.8$\pm$8.3%; $P>0.7$ for all) were not different between groups. REE was reduced in hypothyroid women when compared to the control group in absolute terms (1347$\pm$171 vs 1447$\pm$154 kcal/die; $P<0.05$), when adjusted for LBM (28.3$\pm$2.6 vs 30.5 $\pm$3.0 kcal/kg LBM die; $P<0.02$) and when expressed as the ratio between the measured REE and the expected REE based on the Harris-Benedict Equation (91$\pm$7 vs 95$\pm$7%; $P<0.05$). The respiratory quotient was also different between groups (0.92$\pm$0.07 vs 0.86$\pm$0.06; $P<0.01$), suggesting for impaired fasting lipid oxidation in hypothyroid women.

Conclusions

This study demonstrates that middle-aged, overweight/obese hypothyroid women in L-T_4 replacement therapy, in spite of achieving an optimal serum TSH level, are characterized by altered whole body energy metabolism and substrate disposal supporting the view that additional interventions may be necessary to fully revert the entire set of hypothyroidism-related metabolic alterations.

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P1005

TSH-deficiency is associated with a lower thyroid gland volume in hypopituitaric patients compared to healthy volunteers: a cross-sectional study

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Introduction
Thyroid volume (TV) depends on age, gender, anthropometry, smoking and iodine status. IGF1 plays a role on thyroid growth, as demonstrated in acromegaly and GH-deficiency. Finally, TSH is a well recognised permissive factor for thyroid tissue growth. The aim of the study is to evaluate the long-term effect of TSH-deficiency on TV in hypopituitaric patients compared with healthy volunteers.
Methods
We performed a cross-sectional, controlled study on 58 hypopituitaric patients (36 males, 22 females) with multiple hormonal deficiency (confirmed diagnosis of central hypothyroidism was the main inclusion criteria) (60.0 + 13.9 years), and 244 volunteers (73 males, 171 females) (47.7 + 11.63 years). All subjects underwent thyroid ultrasonography (Siemens Acuson Antares, Philadelphia, USA) performed by the same operator. TV was calculated as the sum of TV of the two lobes, each estimated as: length (cm) × width (cm) × depth (cm) × 0.52.
Results
Age, weight, BMI and body surface area (BSA) were greater in hypopipuitaric patients than healthy volunteers. Thyroid nodules were incidentally discovered at ultrasonography in 17 hypopituitaric (29.3%) and 93 volunteers (38.1%). TV was lower in hypopituitaric patients than in volunteers (6.066 + 5.079 and 9.695 + 3.702 ml, $P < 0.001$). This difference was confirmed also in the subgroup without nodules (mean 4.719 + 3.230 and 9.430 + 3.497 ml, $P < 0.001$), but not when comparing hypopituitaric patients and volunteers with goiter. Finally, TV was lower in hypopituitaric patients without nodules (4.73 + 3.27 ml) than in those with goiter (9.62 + 7.18 ml; $P = 0.003$). These differences were held even after correction of TV for BSA, BMI and age.
Discussion
TV is significantly lower in hypopituitaric patients than in healthy subjects, but the prevalence of thyroid nodules seems to be similar. The reduction of TV in hypopituitaric patients seems to occur only in thyroid glands without nodules. The chronic lack of TSH, as in hypopituitarism, seems to be responsible in vivo for a reduction of TV, but this effect seems to involve mainly the normal thyroid tissue rather than the hyperplastic nodular tissue.

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P1006

Prevalance of GH deficiency in Turkish patients with Hashimoto's thyroiditis: a single center experience

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Background and Aim
Prevalence of Hashimoto's thyroiditis is increasing in Turkey. GH deficiency has been reported to associate with this disorder in several different ethnic population. The aim of this study was to evaluate GH deficiency in the population with Hashimoto's thyroiditis.
Materials and methods
Euthyroid Hashimoto's thyroiditis patients, who admitted to the Department of Endocrinology and Metabolism of Hacettepe University, were included to the study. Demographic and laboratory data of patients were recorded.
Results
One hundred ninety three patients with Hashimoto's thyroiditis were evaluated (17 males, 176 females (8.8 vs 91.2%)). Mean age was 39.94 ± 11.02 (min: 18 years, max: 64 years). There were no co-morbid conditions in any patients. One hundred and eleven of them were using medications containing L-thyroxine (57.5%). IGF1 levels of 179 patients (92.8%) were normal. Fourteen patients had low levels of IGF1. Glucagon stimulation testing in 14 subjects revealed GH deficiency (peak < 3 μg/l) in only one subject. This subject had no response to insulin tolerance test either and she was put GH replacement therapy. Our data reveals the prevalence of GH deficiency in this particular group of Turkish Hashimoto's thyroiditis patients was 0.5%.
Conclusion
We conclude that isolated GH deficiency is rarely observed in Hashimoto's thyroiditis patients. There were diverse outcomes of different studies about GH deficiency in Hashimoto's patients from different ethnic backgrounds (0.4–5%). This preliminary study on this issue demonstrated that GH deficiency is 0.5% this particular group of Turkish Hashimoto's thyroiditis patients.

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P1007

Insulin resistance in patients with thyroid dysfunction and hepatosteatosis

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Introduction
Hepatosteatosis is one of the results of insulin resistance. Thyroid dysfunctions effect on insulin sensitivity. The aim of this study was to evaluate insulin resistance in patients with hepatosteatosis and either hypothyroidism or hyperthyroidism.
Design
A total of 407 patients with hepatosteatosis were included. These patients were further divided into two study subgroups and a control group: 102 subjects with hypothyroidism, 103 with hyperthyroidism and 202 with normal thyroid function in the control group. The institution review board of hospital approved the study. Serum TSH, free T_4, free T_3 concentrations, blood glucose, and insulin levels, serum lipid levels, hepatic transaminases and homeostasis model assessment of insulin resistance (HOMA-IR) were measured. Insulin resistance was calculated according to HOMA-index and compared between the groups. IBM Statistics 20.0 for Windows was used for statistical analysis. χ^2 and ANOVA tests were used for comparing groups.
Results
Average age was 50.8 ± 14.1 years. Male:female ratio was 141:266. Frequencies of insulin resistance in patients with hepatosteatotis and either hypothyroidism, hyperthyroidism, or normal thyroid function were 43, 40, and 48% (P, nonsignificant), respectively. HOMA-IR indices were not statistically different between groups ($P = 0.104$).
Conclusions
Hypothyroidism and hyperthyroidism are not correlated to insulin resistance in patients with hepatosteatosis. We decided that patients with hepatosteatosis already have insulin resistance despite different associated comorbidities. Similar studies in literature did not emphasize on hepatosteatosis in such cases.
Key words
Insulin resistance, hepatosteatosis, hypothyroidism, hyperthyroidism.

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P1008

Vitamin D status in autoimmune hypothyroidism

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Objective
To investigate vitamin D status in patients with autoimmune hypothyroidism.
Methods
The study group consisted of 100 patients with newly diagnosed Hashimato's thyroiditis and 100 subjects as the control group. Parameters of calcium metabolism, thyroid function tests and 25(OH) vitamin D levels were measured.
Results or case presentation
Mean age of the study groups was 33.4 ± 4.8 years with female:male, 72:28. Vitamin D insufficiency/deficiency (25(OH)D < 30 ng/ml) rate was significantly higher in the Hashimoto's group compared with the control subjects (75 vs 20%, $P < 0.0001$). In the Hashimoto group, mean 25(OH) vitamin D levels were significantly lower compared with the control group (12.5 ± 7.0 vs 22.3 ± 7.9 ng/ml, $P < 0.001$). The study group revealed

higher anti-TPO levels in patients vitamin D deficiency 25(OH)D < 20 ng/ml than patients with vitamin D insufficiency group (25(OH)D < 30 ng/ml) (650.4 ± 35.4 vs 340.3 ± 65.4 IU/ml, P 0.001). Serum vitamin D level was inversely correlated with the anti-TPO levels ($r = -0.30$, $P = 0.007$).
Discussion
Vitamin D is involved in immune system and, in particular, on T cell-medicated immunity. Vitamin D receptor is profoundly present in the immature immune cells of thymus and the CD8. Low vitamin D level gives rise to a variety of autoimmune disorders including type 1 diabetes, hypothyroidism.
Conclusion
The higher vitamin D deficiency rates besides lower vitamin D levels in the Hashimoto group together with the inverse correlation between vitamin D and anti-TPO suggest that vitamin D deficiency may have a role in the autoimmune process in Hashimato's thyroiditis.

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P1009

Vascular endothelial growth factor and granulocyte–monocyte colony stimulating factor levels in nodular thyroid diseases

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Introduction
Vascular endothelial growth factor (VEGF) is a specific mitogen for endothelial cells. Granulocyte–monocyte colony stimulating factor (GM-CSF) is a key player in the regulation of steady-state functions. Studies have shown increased levels of VEGF and GM-CSF in benign and malignant tumors. The aim of this study was to evaluate the relation between VEGF and GM-CSF levels and thyroid nodules > 1 cm. and negative for malignancy with fine needle aspiration biopsy (FNAB).
Methods and design
Forty-one female patients with benign euthyroid nodular goiter were enrolled as the patient group and 20 age-matched healthy women without thyroid disease were enrolled as the control group. Age, serum VEGF, serum GM-CSF, TSH, fT_3, fT_4, anti-thyroglobulin (anti-TG) antibody, anti-thyroid peroxidase (anti-TPO) antibody and thyroid volume were compared between the patient and control group.
Results
Only thyroid volume and anti-TG levels were significantly different between the two groups ($P = 0.007$, $P = 0.026$ respectively). Other parameters including VEGF and GM-CSF were not significantly different. Serum VEGF levels were positively correlated with anti-TPO levels in the patient group ($r = 0.325$, $P = 0.036$). No correlations were found between VEGF and other parameters. Serum GM-CSF levels were not correlated with any parameters.
Conclusions
VEGF and GM-CSF were not found to be increased in euthyroid patients with benign nodules and they do not seem to play a role in the development of simple nodular goiter. However, correlation between serum VEGF levels and anti-TPO levels in the patient group may indicate a relation between VEGF and development of autoimmune thyroid diseases as suggested by some authors.

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P1010

Increased TRAb and/or low anti-TPO titers at diagnosis of Graves' disease (GD) are associated with an increased risk of developing ophthalmopathy after onset of GD

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Objective
Patients with low thyroid peroxidase antibodies (anti-TPO) and increased TSH-receptor antibodies (TRAb) at diagnosis of Graves' disease (GD) have been suggested to have an increased risk to develop Graves' ophthalmopathy (GO). The aim was to evaluate if GO development can be predicted.
Design
Observational study with registration of possible GD and GO risk factors.
Methods
Three hundred and ninety nine patients with GD were registered 2003–2008 in Malmö, Sweden and out of these 310 were retrospectively followed up to 6 years. The main outcome measures were anti-TPO titer, TRAb titer, smoking habits, radioiodine treatment, and GO development.
Results
TRAb was assessed with a third generation assay at GD diagnosis in 231 patients. The proportion of patients with GO increased above the median 6.3 IU/l both at diagnosis of GD ($P = 0.001$) and at follow-up ($P = 0.0001$).
The distribution of GO patients anti-TPO above or below 20 kIU/l at diagnosis of GD was similar between groups ($P = 0.239$). However at follow-up anti-TPO < 20 kIU/l was associated with an increased proportion of newly developed GO as compared to the cohort with anti-TPO > 20 kIU/l ($P = 0.018$).
Eighty seven percentage of patients who developed GO after GD diagnosis had TRAb above 6.3 IU/l and/or anti-TPO below 20 kIU/l. The proportion of GO was doubled in GD patients treated with radioiodine but could not explain the described findings.
Conclusions
Anti-TPO < 20 kIU/l and/or TRAb > 6.3 IE/l at the time of GD diagnosis were associated with an increased risk to develop GO after diagnosis of GD.

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P1011

Latent toxoplasmosis: a novel risk factor for autoimmune thyroid diseases in pregnancy?

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Introduction
Latent toxoplasmosis, a zoonosis caused by protozoan *Toxoplasma gondii*, is the most widespread human parasitosis in developed countries (prevalence 20–80%). Upon infection, *T. gondii* stays in human organism lifelong. It has been linked to several autoimmune diseases, including autoimmune thyroid disease (AITD). The aim of our study was to assess the impact of latent *T. gondii* infection on the prevalence of AITD in pregnancy with regard to the pregnancy outcome.
Methods
We assessed the presence of latent toxoplasmosis by using complement-fixation reaction and ELISA-measurement of IgG and IgM antibodies in serum of 1247 consecutive pregnant women in 9th–11th gestational weeks. Two women suspected from acute toxoplasmosis were excluded from the study. We determined serum levels of autoantibodies against thyroid peroxidase (TPOAb) (upper cut-off limit for the first trimester of pregnancy was 143 kU/l) and TSH in the same serum sample. In 592 women, data on pregnancy outcome were available (after exclusion of twin-pregnancies).
Results
The overall prevalence of latent toxoplasmosis among was 22.6%. Women with high elevation of TPOAb (> 500 kU/l) suffered from latent toxoplasmosis more frequently than women with TPOAb < 500 kU/l (36.8 vs 21.8%, $P = 0.007$). This effect wasn't present when the cut-off 143 kU/l was used. Serum levels of TPOAb correlated with the toxoplasma IgG-index of positivity ($P = 0.009$, $r = -0.077$). We didn't find any effect of latent toxoplasmosis on TSH in TPOAb-positive women. However, among the TPOAb-negative ones, those positive for *T. gondii* had significantly lower TSH values (1.051 vs 1.240 mU/l, $P = 0.017$). Women with latent toxoplasmosis didn't have an increased prevalence of complications during pregnancy or adverse pregnancy outcomes.
Conclusions
Latent toxoplasmosis is frequent in pregnancy and it may increase the risk of AITD. However, it is not linked to adverse pregnancy outcomes.

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P1012

Iodine status in women after early miscarriages in the Czech Republic

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Background
Early miscarriages are of multifactorial origin. Subclinical thyroid dysfunction belongs to the common causes. Thyroid dysfunction in pregnancy is caused mainly by thyroid autoimmunity and/or iodine deficiency. The Czech Republic belongs to countries with sufficient iodine intake. The aim of the study was to determine iodine status in women after early miscarriages ($n=183$) and to compare it with randomly chosen age-comparable euthyroid women without previous pregnancy (controls, $n=118$).
Subjects and methods
A total of 183 consecutive women after miscarriage in the 9–12th weeks of pregnancy were included in the study. Within 3 months after miscarriage, we performed a laboratory assessment of urinary iodine concentration (UIC; absorption spectrophotometry) and an evaluation of the thyroid function including thyroid ultrasound. Seventy-two women were supplemented by iodine in previous pregnancy, 73 were not and in 38 the information wasn't available.
Results
Women after miscarriages had a significantly lower median of UIC as compared to controls (92 vs 108.6 μg/l, $P<0.001$). Furthermore, only 72/183 (39.3%) of women after miscarriage had sufficient iodine intake (UIC ≥ 100 μg/l) as compared to 71/118 (60.2%) in controls ($P<0.001$). In the rest of the samples analysed, we noted mild (UIC 50–99 μg/l) and moderate iodine deficiency (UIC 20–49 μg/l) without significant differences among the groups (57.9 vs 39.8% and 2.7 vs 0% respectively). None of the women analysed suffered from severe iodine insufficiency (UIC <20 μg/l). There were no significant differences in UIC between women supplemented with iodine in the previous pregnancy as compared to those who weren't.
Conclusions
Czech women after miscarriages suffer from mild or moderate iodine deficiency significantly more often than healthy women without history of pregnancy. Our data support iodine supplementation in women attempting pregnancy even in iodine-sufficient countries.

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P1013

An open-label, randomized, controlled study of the effectiveness and safety of a high intensity focused ultrasound device compared with observation in patients with non-malignant cold thyroid nodules

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Aim
To compare nodule volume change at 6 months follow-up in patients treated by ultrasound guided high intensity focused ultrasound (US-HIFU) with patients undergoing observation in a simple monocentric, open label, randomized-control study.
Patients and methods
The study was IRB approved and all patients signed an informed consent. 32 patients with 34 benign cold thyroid nodules indicated for surgery were recruited for the study: 21 patients assigned to the HIFU arm and 11 patients/13 nodules to the observation arm. Nodule volume was assessed by US and thyroid function determined by routine assays before and during follow-up. The presence and severity of pressure symptoms and cosmetic complaints were also evaluated. Safety was assessed in all patients during the study period.
Mean age was 49 ± 12 years. Median HIFU duration was 54 min (32–114). The overall median energy deposited was 25 kJ (0.8–79).
Results
At M6, the mean decrease in volume was 1.1 ml (-5.5 to 1.7) and 0.3 ml (-1.1 to 3.7) in the HIFU group and the observed group respectively. ($P=0.0223$). In the HIFU group five patients experienced a volume reduction of over 30% (40.4–82) compared with zero patients in the observed group. At baseline, 66% of the treated patients and 45% of the observed patients had pressure symptoms. At 6M only 23.8% of the treated patients were still having pressure symptoms while no change was recorded in the observed patients. One transitory vocal cord palsy occurred just after HIFU ablation but disappeared within 14 days. After that incident, the device was equipped with a safety feature. Side effects were restricted to mild local pain after the HIFU session. Thyroid function remained unaltered.
Conclusion
HIFU is safe in benign cold thyroid nodule treatment. Its promising impact on nodule volume reduction and pressure symptoms needs to be evaluated on a larger scale.

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P1014

Biochemical testing on wide indication to detect overt hypothyroidism is justified: a population-based case–control study in patients newly diagnosed with overt autoimmune hypothyroidism

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Objectives
Hypothyroid patients report a diversity of symptoms at disease presentation. We studied how useful symptoms are to predict hypothyroidism, and if this may vary between subgroups of patients.
Methods
Patients newly diagnosed with overt autoimmune hypothyroidism ($n=140$) were prospectively identified in a population by linkage to diagnostic laboratory databases, and we concomitantly enrolled individually sex and age-region matched controls ($n=560$). Patients and controls participated in a comprehensive investigational program including the presence of 36 symptoms. Sensitivity and diagnostic odds ratios (DOR, >1 significant, >10 very useful as a diagnostic tool) were calculated. In multivariate models, we identified predictors for presence of each symptom. Subgroup analysis was performed in three age groups (tertiles, group A/B/C = 0–48.1 years/48.1–58.2 years/58.2 years).
Results
13 of 36 symptoms were more frequent (DOR >1) among patients than in controls. DOR was highest for tiredness = 5.94 (3.70–9.60 (95% CI)), feeling unwell = 4.10 (2.57–6.54), hair loss = 4.58 (2.80–7.71), and dry skin = 4.09 (2.73–6.16). Tiredness and feeling unwell were independent of age, but age was a predictor for globulus sensation (only present in the two younger age groups (+/+/−)), swallowing difficulties (+/+/−), anterior neck pain (+/+/−), restlessness (+/+/−), cardiac palpitations (+/−/−), bad mood (+/+/−), constipation (+/+/−), decreased appetite (+/+/−), hair loss (+/+/−), dry skin (+/+/−), and vertigo (+/+/−). Serum T_3 (but not TSH or T_4) was predictor for degree of tiredness, smoking for shortness of breath. No difference was observed between sexes, regions investigated, or alcohol intake level.
Conclusions
Symptom presentation did not differentiate very well between cases and controls, with great overlap especially in elderly people. Interestingly, symptoms were much more associated with age at presentation than with the biochemical degree of hypothyroidism at debut. Biochemical testing on wide indication to diagnose overt hypothyroidism is justified.

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P1015

Associations of (F)T_3 and the ratio FT_3:FT_4 with a cluster of obesity-related cardiovascular risk markers in healthy euthyroid middle-aged men and women

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Background
We have previously shown that a higher BMI and less favorable body composition are associated with relatively higher FT_3 levels (within the euthyroid range) in healthy young men. In this study, we aimed to investigate whether FT_3 and the ratio FT_3:FT_4 are also associated with cardiovascular markers in a healthy euthyroid population of middle-aged men and women.

Methods
Thyroid parameters were measured in 2524 subjects from the Asklepios Study (35–55 years). Analyses were restricted to 2315 subjects (1138 women and 1177 men), not using thyroid medication nor having anti-TPO levels above clinical cut-off or TSH levels outside the reference range (0.27–4.2 mU/l). Thyroid function parameters were determined by automated electrochemiluminescence. Statistical analysis was performed by linear and logistic regression analysis in SPSS.
Results
(F)T_3 and the ratio FT_3:FT_4 were significantly positively related to BMI and waist circumference (both $P<0.0001$) and other elements of the metabolic syndrome, i.e. triglycerides, systolic and diastolic blood pressure and fasting plasma glucose (all P values <0.001). (F)T_3 and the ratio FT_3:FT_4 were also associated with lower HDL-levels ($P<0.001$). The presence of the metabolic syndrome as a whole (both according to the IDF as the ATPIII-criteria) was also related to higher (F)T_3 levels and a higher FT_3:FT_4 ratio (OR = 2, $P<0.0001$). All the associations regarding the metabolic syndrome remained significant after adjustment for sex, age, height, weight or waist circumference, and smoking. The FT_3:FT_4 ratio was further positively associated with obesity-related predictors of cardiovascular events: elevated IL6 (>1.5 pg/ml; OR = 1.5, $P=0.001$) and elevated hs-CRP (>3 mg/l; OR = 1.7, $P=0.001$), even after adjustment for sex, age, height, weight or waist circumference, and smoking.
Conclusion
In healthy euthyroid middle-aged men and women, higher (F)T_3 levels and a higher FT_3:FT_4 ratio are associated with a cluster of obesity-related cardiovascular risk markers. However, no causal inferences can be made from this cross-sectional study.

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P1016

Evaluation of hemostatic parameters in patients with Hashimoto's thyroiditis

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Introduction
Thyroid dysfunctions and autoimmunity may concur in the pathogenesis of hemostasis abnormalities. Hashimoto's thyroiditis (HT) is a frequently chronic autoimmune disease in thyroid gland. The aim of our study were to comparison the hemostatic parameters in patients with HT and control subjects.
Method
A total of 52 women were evaluated. Age, TSH, free T_3, free T_4, anti M, anti Tg, TAFI, TFPI, PAI-1, tPA, PF-4, fibrinogen, plasminogen, protein C and S, freeProtein S, factor V–VII–VIII, vWF, APRC, d-dimer were studied in 34 patients with HT (16 subjects of HT TSH ≥5 mIU/ml, 18 subjects of HT TSH <5 mIU/ml) and 18 controls. Data analysis was performed SPSS. Firstly, HT patients were compared with the control group, then a total of 34 women with HT were divided into two groups according to TSH as TSH <5 and ≥5 mIU/ml.
Results
TFPI ($P<0.05$) and factor V ($P<0.05$) were significantly increased in HT patients compared to the controls. PF-4 were lower in patients with HT TSH ≥5 mIU/ml than in patients with HT TSH <5 mIU/ml and controls. No difference were found between the groups with respect to other hemostatic parameters.
Conclusion
Relation between thyroid function and hemostasis has been studied in the last decades. Although the results of studies are still controversial, the reported studies suggest that subclinical hypothyroidism or hyperthyroidism have an increased hypercoagulable state and overt hypothyroidism has a hypocoagulable state. To the best of our knowledge, there is a few study evaluating many of parameters of coagulation-fibrinolysis system in specifical thyroid disease such as HT, Graves' disease, etc. Therefore, our study may be significant. The results of our study, increased TFPI and factor V, decreased PF-4 in patient with HT, suggest that further studies should be conducted to explain the effect of thyroid functions and its autoimmunity on hemostasis.

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P1017

Comparison of hs-CRP and Fetuine-A levels before and after the treatment of subjects with subclinical hyperthyroidism

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Background
This study was planned to reveal the effect of propylthiouracil treatment on CD40, hs-CRP and Fetuin-A levels of subjects with subclinical hyperthyroidism.
Material and methods
After checking CD40, hs-CRP and Fetuin-A levels of 35 patients with subclinical hyperthyroidism, they were given 50 mg of propylthiouracil tablet (thrice daily). After the 3-month administration, CD40, hs-CRP and Fetuin-A levels were then compared to the levels before treatment.
Results
While hs-CRP and CD40 levels were found normal compared to the healthy controls in SHE patients, Fetuin-A levels were statistically significantly higher (*$P=0.022$). After the treatment, Fetuin-A levels of SHE patients decreased statistically significantly compared to the levels before treatment (**$P=0.026$). CD40 and hs-CRP levels did not revealed a statistically significantly difference compared to the control group and post levothyroxine treatment.
Conclusion
High Fetuin-A levels before propylthiouracil treatment and decrease in these levels after treatment in cases with subclinical hyperthyroidism indicated the possibility of preventing long-term cardiac complications with propylthiouracil treatment.
Key words
CD40, hs-CRP, Fetuin-A, subclinic hyperthyroidism.

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P1018

Thyroid morphofunctional parameters in amiodarone induced thyrotoxicosis compared to endogenous hyperthyroidism

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Introduction
In the interpretation of the thyroid tests in a patient treated chronically with amiodarone (AMD), the effects on the thyroid should be considered. The aim of the study was to evaluate the thyroid parameters in amiodarone-induced thyrotoxicosis (AIT), compared to endogenous hyperthyroidism.
Materials and methods
The study group included 36 patients with AIT (mean age 57.6±7.8 years, 11 women (F), 25 men (M)). The mean duration of AMD-treatment was 26.3±16.4 months. The cases were classified as follows: 10 with type 1 AIT, 13 with type 2, and 13 with mixed type. The control group included 39 patients (F:M, 14:25), with clinical endogenous hyperthyroidism. For type 1 AIT, the control group included: 24 cases with Graves'disease, 2 patients with toxic adenomas, one multinodular goiter, and for type 2 AIT, the control cases were 13 patients with subacute thyroiditis in thyrotoxic phase. The diagnosis was confirmed by a suppressed value of serum TSH (<0.1 mUI/l), associated with high levels of FT_3 and/or FT_4.
Results
The patients with type 1 AIT presented lower thyroid volumes than the controls (30±8.2 vs 34.7±12.3 ml, $P=0.27$), while the type 2 cases presented similar volumes with controls (18.6±5.5 and 17.9±4.1 ml, $P=0.74$). AIT patients presented lower values of serum TSH (0.009±0.013 mU/l) than the controls (0.021±0.037 mU/l, $P=0.067$), although there were no differences among the subtypes of AIT. Mean thyroxine levels were higher in AIT group than the controls (4.48±1.6 and 4.08±1.53 ng/dl, $P=0.29$). FT_3 presented significantly lower values in type 1 AIT (6.62±1.85 pg/ml), than the controls (10.7±5.4 pg/ml, $P=0.02$), while in type 2, it showed similar concentrations (6.2±2.86 vs 7.2±3.77 pg/ml, $P=0.53$).

Conclusion
AIT presents a specific hormonal profile, which reflects the effects of AMD on thyroid hormones metabolism: significantly lower FT_3 ($P=0.01$) and higher FT_4 values ($P=0.29$), as compared to endogenous hyperthyroidism.

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P1019

Differential item functioning of the thyroid-specific quality of life questionnaire ThyPRO

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Objective
Thyroid diseases have been shown to affect quality of life. A thyroid-specific patient-reported outcome (PRO) measuring quality of life, the ThyPRO, has recently been developed and validated using classical clinic- and psycho-metric methods. The purpose of the present study was to apply modern psychometrics to validate the measure as the extent of differential item functioning (DIF) according to sex, age, education and diagnosis, within the ThyPRO.
Methods
Eight hundred and thirty eight patients with benign thyroid diseases (non-toxic goitre, toxic nodular goitre, Graves' hyperthyroidism, Graves orbitopathy and autoimmune hypothyroidism) completed the ThyPRO questionnaire (84 five-point items, 13 scales). Uniform and non-uniform DIF was investigated using ordinal logistic regression, testing for both statistical significance and magnitude (ΔRsq >0.02).
Results
Twenty instances of DIF were found. Seven according to diagnosis, where the goitre scale was the most affected scale, possibly due to differing perceptions in patients with autoimmune thyroid diseases compared to patients with simple goitre. Eight age-related DIFs were found, five of which were in positively worded items, which younger patients were more likely to endorse; one gender-related where women were more likely to report crying, and three according to educational level. The vast majority of DIFs only had minor influence on the scale scores (0.1–2.3 points on the 0–100 scales), but two DIFs corresponded to a difference of 4.6 and 9.8 respectively.
Conclusion
Ordinal logistic regression identified DIF in 17 of 84 items. The potential impact of this on the present scales was low, but the results can assist the planning and interpretation of future clinical trials. Shorter and more clinically implementable versions of the ThyPRO would be of virtue in the future, and items displaying DIF could be avoided when developing such abbreviated scales, where the impact of one items with DIF among fewer items will be larger.

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P1020

Insulin resistance in patients with hypothyroidism or hyperthyroidism without hepatosteatosis

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Introduction
Insulin resistance, refers to decreased response of tissues to insulin, primarily associated with type 2 diabetes and glucose intolerance. Several results were obtained in previous studies about the relation between insulin resistance and thyroid dysfunction. In these studies hepatosteatosis was not an exclusion criteria despite insulin resistance has a key role in the development of it. In this study we aimed to investigate insulin resistance in patients with hypothyroidism or hyperthyroidism without hepatosteatosis.
Design
A total of 340 patients without hepatosteatosis were included. These patients were further divided into two study subgroups and a control group: 106 subjects with hypothyroidism, 104 with hyperthyroidism, and 130 with normal thyroid function in the control group. The institution review board of hospital approved the study. We measured serum TSH, free T_4, free T_3 concentrations, blood glucose and insulin levels, serum lipid levels, hepatic transaminases, and homeostasis model assessment of insulin resistance (HOMA-IR). Insulin resistance was calculated according to HOMA index and compared between the groups. SPSS 19.0 Package Program (SPSS, Inc.) was used for statistical analysis. χ^2 and ANOVA tests were used for comparing groups.
Results
Average age of 340 patients was 41.2 ± 10.9 years. Male:female ratio was 52:288. Frequencies of insulin resistance in hypothyroidism, hyperthyroidism or normal thyroid function were 17, 20, and 24% ($P=0.673$) respectively. HOMA-IR indexes were 1.85 ± 1.18, 1.96 ± 1.54, 2.09 ± 1.79 respectively. The difference was not statistically significant. ($P=0.589$).
Conclusion
In this study, we did not found insulin resistance in patients with hypothyroidism or hyperthyroidism. We concluded that insulin resistance could be associated with comorbidities in patients with thyroid dysfunction in previous studies. In our opinion, hepatosteatosis should be evaluated as a comorbidity in such patients.
Key words
Insulin resistance, hypothyroidism, hyperthyroidism, hepatosteatosis.

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P1021

Should subclinical hypothyroidism diagnosed during pregnancy be treated with long-term L-thyroxine?

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Background
Subclinical hypothyroidism is common in pregnancy affecting about 5% of all pregnant women, and is associated with adverse pregnancy outcomes. There is a general consensus that subclinical hypothyroidism detected during pregnancy should be treated with L-thyroxine (Stagnaro-green *et al*. 2011, DeGroot *et al*. 2012). However, it is unclear whether the treatment should be limited only during the pregnancy or continued long-term. Therefore, we aimed to study whether subclinical hypothyroidism detected during pregnancy is reversible after pregnancy.
Subjects and methods
We analysed TSH, free T_4, free T_3 and thyroid peroxidase antibodies (TPO-Ab) on stored serum samples from 988 women at 28 weeks pregnancy. We carried out same tests on 523 of these women, who had no known thyroid disease or overt hypo- or hyperthyroidism during the pregnancy, on a visit mean (S.D.) 4.9 (1.6) years after delivery.
Results
Subclinical hypothyroidism in pregnancy (defined as TSH >3 mU/l for third trimester; Stagnaro-Green *et al.* 2011, DeGroot *et al*. 2012) was present in 65/523 (12.4%) of women. Of these, 48 (74%) women had normal thyroid function post-pregnancy; only 14 (21.5%) had persistently high TSH (defined as TSH >4.5 mU/l post-pregnancy) and a further 3 (4.6%) were on L-thyroxine. Those with TPO-Ab ($P<0.001$) or TSH above 5 mIU/l ($P=0.03$) in pregnancy were more likely to have persistently elevated TSH or be on L-thyroxine replacement outside pregnancy. 45/523 (8.6%) of patients had isolated maternal hypothyroxinaemia in pregnancy (defined as free T_4 below 10th centile without raised TSH). Only 2 (4.4%) of them had raised TSH outside pregnancy. The proportion of women with positive TPO-Ab more than doubled post-pregnancy compared to that in pregnancy (12 vs 5%).
Conclusions
The majority of cases of subclinical hypothyroidism in pregnancy are transient, so treatment of L-thyroxine in these cases may not be warranted outside of pregnancy.

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P1022

IgG4-related Hashimoto's thyroiditis: an emerging variant of a well known disease

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Introduction
IgG4-related Hashimoto's thyroiditis (HT) is a very recently reported entity, characterized by thyroid inflammation rich in IgG4-positive plasma cells associated with marked fibrosis. It may be part of the systemic IgG4-related

sclerosing disease and is associated with younger age, lower female:male ratio, higher levels of thyroid autoantibodies, low echogenicity on ultrasound (US), rapid progress requiring surgery and more subclinical hypothyroidism, when compared with non-IgG4 HT.

Case report

A 56-year-old man presented with a 4-month history of progressive neck swelling, dysphagia, and weight loss of 7 kg. Cervical palpation identified an enlarged, hard and painless thyroid gland. Laboratory testing revealed: increased ESR, 81 mm/h (<31) and CRP, 8.2 mg/dl (<1), elevated TSH, 19 mIU/l (0.1–4), normal free T_4, 0.99 ng/dl (0.93–1.7) and very high levels of anti-Tg, >4000 IU/ml (<40) and anti-TPO, >600 IU/ml (<35). US demonstrated an enlarged and heterogeneous thyroid gland, with a substernal component, both lobes with maximal dimension >10 cm and two hypoechoic nodules with 3.2 and 2.2 cm located on the isthmus and right lobe, respectively. US-guided fine needle aspiration cytology was performed and the material removed from both nodules was consistent with lymphocytic thyroiditis. The patient was submitted to total thyroidectomy with no surgical complications. Histological findings showed lymphoplasmacytic infiltration, lymphoid follicles with germinal centers and marked fibrosis limited within the thyroid capsule; an increased number of IgG4-positive plasma cells was found by immunohistochemistry. One month after surgery, serum IgG4 concentration was high-normal, 165 mg/dl (3–201). Symptoms relief and reduction in laboratory inflammatory parameters were noticed. Thyroid function was controlled with levothyroxine.

Conclusions

We report a typical case of IgG4-related HT. The presentation form suggested the diagnosis, which was confirmed based on histological data. Our case highlights this new variant of the well known HT, and helps physicians in recognizing its main clinical features.

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P1023

Prevalence of thyroiditis in ankylosing spondylitis patients

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Objective

We aimed to evaluate the prevalance of thyroiditis in ankylosing spondylitis patients in this study. We investigated the role of serum procalcitonin as a chronic inflamatory marker in ankylosing spondylitis.

Methods

Sixty-seven patients who diagnosed as ankylosing spondylitis according to American College of Rheumatology (ACR) criteria and 57 healthy controls were included in our study. Serum levels of procalcitonin, TSH, free triiodothyronine (FT_3), free thyroxine (FT_4), anti thyroglobulin (anti-TG) and antithyroid peroxidase (anti-TPO) were measured in both groups. The presence of tyroiditis and nodules were evaluated by ultrasonography of the thyroid gland. The disease activity of ankylosing spondylitis were evaluated by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).

Results

There were no significant differences in terms of tyroiditis between patient and control groups. It was not detected correlation between serum procalcitonin level and BASDAI in patient group. It was shown that there was no correlation among serum procalcitonin levels, the presence of nodules, and thyroiditis. But, there was a correlation between thyroiditis and serum procalcitonin levels in control group ($P<0.05$).

Table 1 Demographic and clinical parameters of the groups.

	Ankylosing spondylitis group ($n=67$) Mean±S.D.	Healthy control group ($n=57$) Mean±S.D.
Age (years)	38.7±10.4	38.3±12.0
Gender		
Male (*n* (%))	55 (82.1)	46 (80.7)
Female (*n* (%))	12 (17.9)	11 (19.3)
Tyroiditis		
Present (*n* (%))	23 (34.3)	13 (22.8)
Absent (*n* (%))	44 (65.7)	44 (77.2)
Nodules		
Present (*n* (%))	12 (17.9)	4 (7)
Absent (*n* (%))	55 (82.1)	53 (93)

Table 2 Evaluation of serum procalcitonin levels between the groups.

	Procalcitonin levels in patient group with ankylosing spondylitis	*P* value	Procalcitonin levels in healthy control group	*P* value
Tyroiditis present	0.09±0.03	>0.05	0.14±0.03	**0.004**
Tyroiditis absent	0.08±0.04		0.05±0.25	
Nodules present	0.09±0.05	>0.05	0.04±0.02	>0.05
Nodules absent	0.08±0.03		0.07±0.13	

Conclusion

Although some studies in the literature that showing a significantly higher prevalence of thyroid otoimmunity in patients with spondyloarthritis, we could not show such a relationship in our study. Furthermore, serum procalcitonin concentration was elevated in some cases of tyroiditis. However, it was not shown a relation between procalcitonin and ankylosing spondylitis which was one of chronic inflammatory diseases.

Key words

Ankylosing spondylitis, procalcitonin, thyroiditis.

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P1024

The study of gastrointestinal hormones in patients with hypothyroidism of middle-aged and elderly

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The purpose of study was to examine the content of gastrointestinal hormones in patients with hypothyroidism.

Objectives and methods

We investigated of 37 patients, which hypothyroidism, at the age from 23 to 65 years. The diagnosis of hypothyroidism was confirmed by measurement of thyroid hormones and TSH in serum. Immunohistochemistry in gastrobiopsies of the antrum of patients studied gastrin, serotonin, melatonin. The primary antibodies used commercial antibodies to serotonin, gastrin, melatonin (Dako, Glostrup, Denmark, caption 1:50). Measurements were conducted in five fields of view (objective with increase in 40) in three slices of the examined organ.

Results

In the group of the patients in whom no biopsy serotonin and melatonin in the blood TSH level was 19.9±3.8 mIU/l, the level of free thyroxine (T_4) of 7.8±2.3 pmol/l, total T_4 8.1±3.8 ng/ml. In the group of patients whose biopsy visually determined serotonin in the blood TSH level was 14.38±2.4 mIU/l, the level of free T_4 of 8.1±2.3 pmol/l, total T_4 47±4.9 ng/ml. Only in biopsies of patients with hypothyroidism were detected all young gastrointestinal hormones: gastrin-secreting cells (G-cells) 5.6±3.7%, serotonin-secreting cells (EC-cell) 3.7±1.3%, melatonin-producing cells 1.3±0.6%. Given results testify about the disturbance in the system of local hormonal gastrointestinal regulation, close interrelation of disturbances (GIG) with the vegetative nervous system.

Conclusion

Thus, the results indicate a possible violation of local gastrointestinal hormonal regulation in patients with hypothyroidism with middle-aged and elderly.

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P1025

The study of levels erythropoietin and proinflammatory cytokines in patients with hypothyroidism of different ages

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Objectives
The aim of study was to examine the levels erythropoietin in hypothyroidism with anemic syndrome of different ages.
Methods
We investigated 52 patients with hypothyroidism with irondeficiency normochromic and hypochromic anemia. Among them were 21 patients between ages from 21 to till 45 years old and 31 over 45 years of age. Examination included total blood, in the blood levels of thyroid hormones, serum iron, erythropoietin, ferritin by standard methods.
Results
As a result it was found that 38% of patients with Hb levels of the blood was below 70 g/l and 62% of patients with Hb levels was below 90 g/l between ages from 21 to till 45 years old. So study revealed that patients over 45 years of age 19% have Hb levels of the blood was below 70 g/l and 81% patients below 90 g/l. The level of erythropoietin in blood plasma in 71% of patients was below 5 ng/ml. At 42% of patients level ferritin was below 20 ng/ml that proves depletion of iron levels. The studying interleukins of patients with hypothyroidism showed an increase in proinflammatory cytokines IFNγ up to 7.15 ± 2.3 pg/ml and IL1β 11.4 ± 5.2 pg/ml that testifies to suppression of the level of erythropoietin by interleukins. In the analysis of thyroid function in patients with hypothyroidism with anemia it was found that the level of TSH was within 23.7 ± 2.8 mIU/ml and free T_4 7.9 ± 1.3 pg/ml, antibodies to thyroid peroxidase 327.5 ± 62.7 IU/ml.
Conclusion
Thus, in hypothyroidism with anemia, a change of proinflammatory cytokines and decreased levels of erythropoietin and levels. And in patients with hypothyroidism with anemia a younger age discovered severe anemia.

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P1026

Acute renal failure as the first presentation of severe hypothyroidism

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Introduction
Renal impairment associated with hypothyroidism without any other clear underlying cause is relatively rare. We report a case with severe hypothyroidism admitted with acute renal failure.
Case report
A 72 years old female patient was addmitted to the emergency department with progressively worsening complaints of fatigue, somnolence, and swellings all over her body for the last 3 weeks. Initial laboratory examination revealed acute renal failure (BUN: 150 mg/dl, creatinine: 5.03 mg/dl) after which she was hospitalized. Past history disclosed no previous thyroid disease, thyroid medication, or thyroid surgery. History about any renal disease was also unrevealing. She did not receive any statin or fibrate therapy. She only received ramipril for hypertension for about 15 years. Physical examination was notable for stuporous and dysoriented state, periorbital, facial, and lower extremity edema. She was not hypothermic or hypotensive. Further laboratory evaluation showed severe hypothyroidism (TSH >100 mIU/ml, fT_4 <0.40 ng/dl, fT_3 <1.00 pg/ml) and high creatine phosphokinase levels with normal MB fraction suggesting rhabdomyolysis. Thyroid autoantibody levels were positive. Autoantibody screening for systemic autoimmune disease and complement levels were normal. Urinalysis was negative for blood and protein in the urine. Ultrasonographic evaluation of urinary system and doppler ultrasonography of arteriovenous system of the kidneys were unremarkable. A randomly measured cortisol level was 20.2 μg/dl and she was started on glucocorticoid therapy. Replacement with levothyroxine was started soon after with mild increments under close cardiac monitorization. Supportive therapy included parenteral hydration and ampiric antibiotics. Her symptoms improved, BUN and creatinine levels returned to normal after seventh day of treatment. Glucocorticoid therapy was rapidly tapered and stopped after second cortisol measurement (11 μg/dl morning).
Conclusion
Hypothyroidism may be a cause or triggering factor of acute renal failure. Thyroid function tests may become part of the routine list to go through as a cause of acute renal failure.

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P1027

Thyroid diffuse lipomatosis: a rare and benign disease

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Introduction
Diffuse lipomatosis of the thyroid is a very rare disease, characterized by extensive infiltration of thyroid parenchyma by mature adipose tissue. It is not accompanied by accumulation of amyloid fibrils.
Clinical report
Male, 47 years old, followed in Endocrinology by hypothyroidism, since 13 years old, medicated with levothyroxine, with unremarkable growth and pshicomotor development. In 2002, thyroid echography showed a solid and heterogeneous lesion (6.6×3.3 cm size), suggestive of a lipomatosis formation. Fine needle biopsies were always inconclusive. In 2010, CT and MRI showed the same cervical lipomatosis lesion (15×6, 5×4, 9 cm), occupying the entire thyroid anatomic region and extending till the retropharyngeal space.
Micro biopsy revealed: 'fibro-fatty tissue surrounding regular thyroid follicles'. Surgical excision of the neck mass showed an extensive infiltration of the thyroid parenchyma by mature adipose tissue without atypia. These morphological findings and the history of subtotal thyroidectomy by thyroid nodule at 3 years of age with identical histological characteristics have allowed the diagnosis of diffuse lipomatosis of the thyroid gland.
Conclusion
The pathophysiology of adipose tissue infiltration in the thyroid gland is not clear. The definitive treatment is surgery and most of the lesions are benign. The prognosis is favourable.

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P1028

Regression of ophthalmopathic exophthalmos in Graves' disease after thyroidectomy: an observational study in a surgical series

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Background
Ophthalmopathic exophthalmos is reported to favourably regress after total thyroidectomy compared to radio-iodine or antithyroid drug therapy. In this context, we present our experience based on a surgical series of Graves' disease.
Materials and methods
This is a prospective study of 15 patients of Graves' disease associated with ophthalmopathic exophthalmos. Preoperative and monthly postoperative evaluation of exophthalmos was done with Hertel's exophthalmometer with a minimum follow up of the cohort was 12 months.
Results
F:M ratio was 12:3 and mean age = 33.4 years (18–55). Exophthalmos was bilateral in 13 and unilateral in two patients. Exophthalmos regressed in 12 patients at mean follow-up of 8 months (2–14) and was static in 3. Mean regression of exophthalmos was 2.2 mm (1–5). The regression was statistically significant at P value = 0.04.
Conclusions
Graves' disease associated exophthalmos regresses in 73% of cases with significant symptomatic relief, after thyroidectomy. Total thyroidectomy appears to be an ideal management for Graves' disease associated with opthalmopathic exophthalmos.
Key words
Graves' disease; Ophthalmopathy; Exophthalmos; Total thyroidectomy; Regression.

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P1029

Vitamin E supplementation in the treatment of Graves' disease

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Hyperthyroidism in the course of Graves' disease leads to intensification of oxidative processes resulting in increased oxidative stress.
Objectives
The effect of supplementation with vitamin E was monitored on the speed of attaining euthyroidism and on the oxidative stress parameters in patients with Graves' disease, treated with thiamazole.
Patients and methods
We examined 43 hyperthyroid patients and 12 euthyroid healthy controls. Patients were divided into two groups according to the treatment: Thiamazole + Metoprolol group and Thiamazole + Metoprolol + vitamin E (Vit E) group. Malondialdehyde (MDA), the marker of lipid peroxidation, carbonyl proteins and reduced glutathion (GSH) were determined from the serum, twice, before the treatment and after 4 weeks of therapy.
Results
Patients who received supplementation with vitamin E in addition to therapy with thiamazole (Group 2) attained euthyroidism faster than the patients treated with only thiamazole (Group 1). The marker of lipid peroxidation, MDA and GSH (reduced glutathione) decreased significantly in the Graves' disease supplemented patients, compared to those who were treated with thiamazole alone.
Conclusion
The results of the study clearly indicate that supplementation with antioxidants in the treatment of Graves' disease is justified, particularly those containing vitamin E. Nutritional antioxidants as pharmacological compounds may represent an innovative therapeutic approach to hyperthyroidism as a combined treatment with antithyroid drugs.

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P1030

Radioiodine treatment of hyperthyroidism in the elderly

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Introduction
Treatment of hyperthyroidism in older adults is crucial to reduce its morbidity and mortality. Radioactive iodine has been widely used in this age group due to its efficacy, safety and cost-effectiveness.
Aim
To determine the efficacy of ^{131}I for treatment of hyperthyroidism in the elderly.
Methods
Retrospective study of 86 hyperthyroid patients aged $\geq$65 years who performed radioiodine therapy. We evaluated the following parameters: gender, age, thyroid disease, clinical, and laboratory situation at 1, 3, and 5 years after treatment. We excluded 22 patients who didn't have a minimal follow-up of 1 year.
Results
We evaluated 64 patients (89% females) with a mean age ($\pm$S.D.) of 74.4$\pm$6.4 years (range 65–89 years) at the time of radioiodine treatment. Twenty-eight patients (44%) had toxic multinodular goiter, 27 (42%) had toxic adenoma and 9 (14%) had Graves' disease. We documented hyperthyroidism, subclinical in most cases, in 27% (17/63), 12% (5/42), and 6% (2/32) of patients at 1, 3, and 5 years after radioiodine therapy, respectively. The prevalence of hypothyroidism was 10% (6/63), 24% (10/42), and 38% (12/32) at 1, 3, and 5 years after treatment with ^{131}I respectively. Euthyroidism was observed in 63% (40/63), 64% (27/42), and 56% (18/32) after 1, 3, and 5 years of follow-up, respectively. Seven patients (11%) needed two and one patient (1.6%) needed three doses of radioiodine to solve the hyperthyroidism. One patient underwent surgery. There were no significant complications due to radioiodine therapy.
Conclusion
Radioiodine therapy has proved to be effective and safe to control hyperthyroidism in this age group. The cell necrosis induced by radioiodine occurs gradually hence its effect may not be achieved immediately.

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P1031

The influence of subclinical hypothyroidism on glucose levels

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Background
Recently many authors are questioning the existing of elements of metabolic syndrome (MetSy) in patients with subclinical hypothyroidism (SH). In this cross sectional study we observed glycemia, as an element of MetSy, in newly diagnosed patients with SH.
Materials and methods
Seventy females participated in study. Among them there were 50 patients with SH, mean age 58.3 ($\pm$8.53), and 20 controls without SH, mean age 51.1 ($\pm$6.79). We determined next paramethers: TSH, FT_4, anti TPO-antibody (by ELISA method), and glycemia, insulinemia, HgA1c, and performed oral glucose tolerance test to all who did not already have diabetes mellitus type 2. We measured body waist, weight, and height. We calculated BMI and HOMA indexes. For statistical calculations we used EXCEL, Med-Calc and SPSS Programs.
Results
The patients were older then controls, they had higher levels of waist (W) and BMI. In the group of patients we found significantly higher percent of diabetes mellitus type 2 (DM2), 36%, and among them 14% newly diagnosed DM2. In the control group there were no DM. In the patients group, as expected, we found higher levels of TSH, anti TPO-Ab, and lower levels of FT_4. Concentraion of glucose and HgA1c were higher among the patients. There were statistical significantly difference between the groups in waist $F=5.64$, $P=0.020$; glucose $F=4.40$, $P=0.040$; HgA1c $F=4.90$, $P=0.030$; TSH $F=116.62$, $P<0.001$; FT_4 $F=5.97$, $P=0{,}017$; and anti TPO-Ab $F=63.23$, $P<$ 0,001. We did not find statistical significance in HOMA and insulinemia. We found that there was positive correlation between the concentration of glucose and TSH ($R=0.304$, $P=0.032$) in patients group, but not in the control group. We did not find positive correlation between glucose and FT_4 in both groups.
Conclusions
Considering the results of our study we recommend determination of glucose levels (and, if necessary OGTT) in every patient whom we diagnosed SH.

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P1032

Coexistent toxic adenoma and Riedel thyroiditis: a case report

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Riedel thyroiditis is a rare chronic inflammatory disease of the thyroid. It is characterized by replacement of normal parenchyma with dense fibrotic tissue. Peripheral tissues might also be affected and this may cause airway obstruction, dysphagia, recurrent laryngeal nerve palsy and hypoparathyroidism. We report a patient with toxic adenoma previously treated with radioactive iodine and histopathologically confirmed Riedel thyroiditis.
Case
A 61 years old male patient was referred because of subclinical hyperthyroidism. He did not have any obstructive symptoms. In physical examination, a 3$\times$2 cm nodule was detected in the left lobe. Serum TSH, fT_3, fT_4 and thyroglubulin levels were 0.047 μIU/ml (0.4–4 μIU/ml), 2.04 pg/ml (1.57–4.71 pg/ml), 1.03 ng/dl (0.85–1.78 ng/dl), and 6.31 mg/dl (1.15–35 mg/dl) respectively. Antithyroid peroxidase, antithyroglobulin and thyroid stimulating antibodies were negative. Thyroid ultrasonography revealed a 15$\times$20$\times$28 mm isoechoic nodule located in superior and mid portions of left lobe. Ultrasonographically the nodule had a thin hypoechoic halo, cytic degeneration areas and macrocalcification. Thyroid scintigraphy showed an active nodule with extranodular suppression of thyroid parenchyma. Uptake was 10% after 4 h and 25% after 24 h of I^{131} administration. The nodule was evalueated with fine needle aspiration biospy and cytology was benign. The patient was treated with 20 mci radioactive iodine for toxic adenoma. In follow up, since nodule diameter increased significantly after 6 months, total thyroidectomy was performed. In histopathological examination, there was marked fibrosis in stroma and some atrophic glands in thyroid tissue. Fibrosis was extending to the surrounding fat tissue and focal chronic inflammatory cells

were observed around middle sized veins. With these findings, the patient was diagnosed to have Riedel thyroiditis.
Conclusion
Riedel thyroiditis is a very rare disease of the thyroid gland. To our knowledge, this is the first case with coexistent toxic adenoma and Riedel thyroiditis reported in the literature.

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P1033

Internal jugular vein thrombosis and intensely hypermetabolic thyroid on ^{18}F-FDG PET/CT

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Internal jugular vein thrombosis (IJVT) is generally related to central venous access devices (CVADs) because of endothelial trauma or inflammation. Other reported causes include neck surgery complication, malignancies, deep neck infections, intravenous drug abuse, ovarian hyperstimulation syndrome and hypercoagulable states.
We report the case of a 47-year-old woman presenting with acute neck pain, local swelling and fever. Neck ultrasound with Doppler studies showed a goiter of 48 ml with a heterogeneous parenchyma and a left internal jugular vein thrombosis of recent appearance. Blood tests revealed elevated inflammatory markers, overt hyperthyroidism (TSH <0.04 µUI/ml, FT_4 3 ng/dl, FT_3 7.9 pg/ml) and high serum thyroglobulin (183 ng/ml). Anti-thyroid autoantibodies were absent. Technetium-99m pertechnetate scintigraphy showed a very low uptake, suggestive of thyroiditis. Nonsteroidal anti-inflammatory treatment was administered, in addition to low-molecular weight heparin. The most common causes of IJVT were investigated. A complete screening of inherited and acquired prothrombotic factors was negative. ^{18}F-FDG PET/CT showed an unusually intense and diffuse uptake in thyroid gland, with extension to surrounding tissues and multiple bilateral adenopathies, strongly evocative of a thyroid lymphoma. A progressive decrease of neck pain and swelling was observed, and a wait and see attitude was decided. After 6-month delay, the complete normalisation of ^{18}F-FDG PET/CT and neck ultrasound was observed, confirming the diagnosis of subacute thyroiditis. Very limited data are reported in literature on the correlation between deep venous thrombosis and subacute thyroiditis. Some reports on sinus or cerebral thrombosis after thyrotoxicosis suggests that hyperthyroidism could be associated with a hypercoagulable state. The exact mechanism is still unknown. This case shows that in a patient with subacute thyroiditis presenting with persistent neck pain and swelling, IJVT must be ruled out. We discuss the diagnostic challenge represented by intense and diffuse hypermetabolic activity in thyroid gland at ^{18}F-FDG PET/CT.

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P1034

Comparison of pre- and post-levothyroxine hs-CRP and fetuin-A levels in subclinical hypothyroidism

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Background
The objective of this trial is to determine pre- and post-levothyroxine treatment levels of inflammation markers, high sensitive-C reactive protein (hs-CRP) and fetuin A in cases with subclinical hypothyroidism.
Material and methods
A total of 32 patients with a diagnosis of subclinical hypothyroidism and a control group of 30 healthy individuals were tested for hs-CRP ile fetuin A levels, followed by administration of 50 µg levothyroxine in the patient group for 3 months. During post-treatment stage, hs-CRP and fetuin A levels in the patient group were re-assessed and compared with pre-treatment values.
Results
Pre-treatment levels of both hs-CRP and fetuin A were observed to be higher than the control group. The decrease in hs-CRP levels during post-treatment stage did not reach a statistically significant level (*P*: 0.440). However, decrease observed in post-treatment fetuin-A levels was found to be statistically significant (*P*: 0.012).
Conclusion
The decrease in fetuin A levels in subclinical hypothyroidism cases indicates that levothyroxine treatment exerts antiinflammatory and antiapoptotic actions. Even though the decrease in hs-CRP level is statistically insignificant, it is estimated to reach significant levels with sustained treatment.
Keywords
Subclinical hypothyroidism, hs-CRP, fetuin A, levothyroxine.

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P1035

The natural history of subclinical hyperthyroidism: a single centre experience

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Introduction
The possibility of progression of subclinical hyperthyroidism (SH) to overt hyperthyroidism (OH) is a critical point in deciding whether to treat this situation. The aim of this study is to evaluate the rate of progression of SH to OH and the factors influencing this outcome.
Methods/design
This is a retrospective study at an endocrine referral service in Northern Greece. Data from patients with SH diagnosed between 1996 and 2011 were extracted from the department's electronic database. Patients with past history of thyrotoxicosis treated with radioiodine, surgery or antithyroids, thyroiditis, pregnancy/*postpartum* state, severe illness and pituitary disease were excluded.
Results
Forty patients (37 females/3males) aged 53.9 ± 14.3 years (range 25–76) were included. Mean TSH values at baseline were 0.19 ± 0.13 mIU/l. In 18 patients (45%) the diagnosis was subclinical Graves' disease (GD), 13 (32.5%) multinodular toxic goitre (MTG) and 9 (22.5%) toxic adenoma (TA).
Progression to OH was seen five patients, (12.5%), in two (11.1%) with GD, two (22%) with TA and one (7.7%) with MTG, during a mean follow-up time of 34.2 ± 21.3 months. OH was observed in 4/36 (11.1%) at 1 year, 1/23 (4.5%) at 2 years, 0/15 (0%) at 3 years and 1/17 (5.9%) at 5 years. Three of five patients with SH progressed to OH in 6 months.
Interestingly, eight patients at 1 year (22%) normalized thyroid function, 5 (23%) at 2 years, 4 (27%) at 3 years and 6 (35%) at 5 years of follow-up. Five of seven (71.4%) with available follow-up data remained euthyroid during follow-up.
Conclusions
GD was the most common etiology of SH. A very small proportion of patients progressed to OH over 5 years. However, a considerable amount of patients with SH returned to normal thyroid function either for the remainder of follow-up, or only to return to SH state.

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P1036

Morpho-functional features of rabbit's thyroid gland with its autotransplantation in the thigh muscle

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The aim of our study was to determine the possibility of engraftment and function of the native thyroid tissue in its autotransplantation in the thigh muscle in rabbits. The experiment included 10 animals species Chinchilla Breed at the age of 12–18 months – five females and five males. In order to simulate the animals primary postoperative hypothyroidism animal extrafascial thyroidectomy was performed, and then on the inside of the left thigh of the neurovascular bundle formed femoral canal in the hip adductors, which fits within the thyroid tissue. Thyroid hormone levels before surgery was 1.0485 ± 0.05 pg/ml for T_3 and 1.2082 ± 0.05 pg/ml for T_4. During the first 3 weeks, showed a decline in hormone levels, reaching a minimum for 4th week – 0.8344 and 1.085 pg/ml for T_3 and T_4, respectively. Restoring the original hormone levels was observed in four rabbits, 2 months, in three rabbits – 3 months. Two rabbits were followed for 6 months after surgery, and thyroid hormone levels in 2 months remained above average, slightly decreasing after 3 months. In the early postoperative period (1 week) two rabbits

died of pulmonary complications, in the late postoperative period – one (uterine bleeding). The conclusion of the experiment was conducted according to international standards in the period of 3 months (five rabbits) and 6 months (two rabbits). Histological examination of material from the site of transplantation showed a massive proliferation of connective tissue and fat, increased vascularization of the muscles in the area of operation. However, the thyroid tissue not visualized either macroscopically or with standard staining with hematoxylin-eosin and pikrofuksin.

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P1037

Sexual dysfunction in women with thyroid pathology

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Introduction
Sexual dysfunction is a particular pathology with relative high prevalence, often underestimated in general population. It's manifest as an impairment of sexual desire, orgasm, sexual excitement or pain related to intercourse. Although often recognize a psychogenic cause, sexual dysfunction are found also in various systemic diseases more or less related to changes of sexual hormones. Thyroid pathology, particularly hyper and hypothyroidism, with high frequency in women, causes problems with sexual performance related with changes of unbound sex hormone and individual response to organic disease.
Materials and methods
Were analyzed 20 women with hyperthyroidism and 30 women with hypothyroidism, admitted in Endocrinology Clinics of Cluj-Napoca. Through history were established menstrual cycle disorders and disorders of sexual function quality based on a questionnaire completed by each patient voluntarily. Also hormonal determinations were performed to examine thyroid and ovarian function.
Results
In the group with hyperthyroidism we obtained a frequency of 40% of sexual dysfunction. Regarding menstrual cycle disorders, there was a relatively uniform distribution, without significant quantitatively changes between the group with amenorrhea, bradimenorrhea and polymenorrhea. In 20% of cases were reported no menstrual disorders.
In the hypothyroidism group we achieved a higher frequency of sexual disorders 53.3%, compared with the group with hyperthyroidism, with the same uniform distribution in terms of menstrual disorders. In 20% of cases with sexual dysfunction, the disorders of orgasm, arousal and sexual desire were reported only after the thyroid disease debut.
Conclusions
Sexual dysfunction is more common in women with hypothyroidism compared to those with hyperthyroidism.
For both groups most affected disorders of sexual dysfunction were orgasm and sexual arousal.
In hypothyroidism, low FT_4 level and increased PRL influence sexual activity in women.
Menstrual cycle disorders are more common in the group with hyperthyroidism, directly related to testosterone surge.

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P1038

Relationship of benign thyroid disorders with histological alterations of breast tissue. (Preliminary results of an observational study conducted in Greece)

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Introduction
The association of thyroid disorders including autoimmune thyroiditis with the risk for breast cancer is controversial (1–4). A recent study found that triidothyronine (T_3) levels are positively associated with breast cancer specific mortality (5). Experimental studies have shown that T_3 mimics the effects of estrogen in human breast cell lines and induces the expression of progesterone receptors (6).
Purpose
The relationship of benign thyroid diseases (BTD) with histopathological alterations of breast tissue diagnosed by biopsy.
Methods
Ninety-three women were followed in an outpatient clinical center for breast diseases (23 premenopausal, 64 postmenopausal and 6 in perimenopausal period). Seventy of them were diagnosed for breast cancer (BC), 20 for benign breast disease (BBD) and 3 for atypia. The history of BTD was determined by medical records and personal interviews. Patients were divided into four groups based on their thyroid functional or morphological status (autoimmune hypothyroidism (10 patients), non autoimmune hypothyroidism (5 patients), hyperthyroidism of any cause (3 patients) and nontoxic goiter (15 patients)). Thyroid hormones functional tests and anti-TPO Ab titres were measured before any surgery or treatment (chemotherapy or radiotherapy).
Results
The overall prevalence of BTD was 22 in 70 (31.4%) women with BC and 8 in 20 women (4%) with BBD ($P=0.592$). No association to BC or BBD was found when studying each type of BTD separately ($P=0.135$). These results were independent to thyroid treatment ($P=0.594$). Mean fT_3, fT_4 and TSH concentrations in women without treatment for BTD, showed no difference between BC and BBD patients ($P=0.249$, $P=0.187$, $P=0.209$ respectively). FT_3 levels were not associated to the concentration of estrogen or progesterone receptors of breast cancer cells ($P=0.683$). Anti-TPO Ab (>50 U/ml) were not associated to specific histopathological patterns ($P=0.641$).
Conclusions
Preliminary data show that although overall prevalence of BTD is increased in BC patients compared to patients with BBD the difference was not statistically significant probably due to the small sample.

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P1039

Clinical outcomes in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine

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The most common cause of hyperthyroidism in young patients is Graves' disease (GD). It is an auto immune disorder caused by TSH-receptor-stimulating auto antibodies.
Treatment options for GD include anti-thyroid drugs (ATD), radioactive iodine (RAI), and surgery. The treatment modalities used vary in different parts of the world.
Objectives
To compare the effect of different treatment and to determine the profile of patients who undergo such and such a treatment modalities outcome.
Methods
A retrospective study between 2007 and 2012 at the central hospital of the army. 100 GD patients were included and received different treatment.
Results
We treated 100 patients (63 men and 37 women) the average age was 33 years. 42 patients received ATD, 17 patients randomized to surgery, and 35 patients were treated with RAI. Subjects were followed for mean of 31 months (range, 1–69 months) for the following clinical outcomes: 73% of patients treated by ATD experienced persistent/recurrent hyperthyroidism. 100% of patients who undergo surgery became free of disease after total thyroidectomy without recurrence. Complication rate was 18%. 81% were successfully treated (hypothyroid or euthyroid) after a single dose of RAI with no acute complication.
Conclusion
In our opinion the medical therapy (ATD: 24 months) is not a very performed therapy in our country taking into account our socioeconomic context. Thyroidectomy can be performed with low complication rates and there is no risk of recurrence if total thyroidectomy is performed. But not indicated for all patients. The RAI is a safe and effective treatment for GD. It is relatively inexpensive. For our young military patients it is the ideal therapy for a speedy recovery with no acute complication.

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P1040

Thyroid hormone elevates IL6 tissues content and causes hypoleptinemia

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Oxidative stress is regarded as a pathogenic factor in hyperthyroidism. IL6 has been described as acting as a protective intrahepatocellular against oxidative stress and mitochondrial dysfunction. On the opposite way leptin has been shown to increase oxidative stress. The impact of thyroid hormones on circulating cytokines levels has been demonstrated with conflicting results both in human and rats. Most of the studies concerned hyperthyroidism showed increased levels of IL6 compared either with controls. Our purpose was to determine the effect of hyperthyroidism and hypothyroidism on the concentration of IL6 in different tissues and leptin serum concentration. Male Wistar rats (60 days of age) were randomly distributed into three different groups: C – control group that received daily i.p. injection of vehicle; Hyper – hyperthyroid rats group that received daily i.p. injection of T_4 (50 μg/rat); Hypo- hypothyroid rats group that received daily i.p. injection of propylthiouracil (50 μg/rat). 21 days after treatment, the animals were killed and serum, retroperitoneal adipose tissue, EDL and soleum muscles, and liver were collected. Tissues IL6 level, body weight gain, serum lipid profile, leptin and insulin were determined. The serum lipid profile and body weight gain and food consumption were similar among groups. All analysed parameters were similar between Hypo and C. However, the Hyper showed: increase in liver and EDL IL6 level; decrease in serum leptin levels; and elevation in insulin and glucose serum concentration. These results demonstrated that 21 days of propylthiouracil treatment did not affect the analysed parameters. However, the increase in tissues IL6 content and decrease leptin levels in T_4 treated group could be a compensatory modulation of the oxidative stress caused by hyperthyroidism state.

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P1041

The determination of nitric oxide levels in treatment-naïve hypothyroid females: pilot study

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Background
Hypothyrosis is an atherosclerosis-promoted condition. On the other hand, nitric oxide (NO) has antiatherogenic cardioprotective properties. By some Doppler ultrasound parameters (intima-media complex thickness (IMCT) and systolic velocity (SV)), one can point out the level of atherosclerosis presence in cardiovascular system out of histopathology.
We primarily determine NO levels in studied population and then examine their influence on mentioned Doppler ultrasound parameters.
Methods and design
In this pilot study, 28 females were divided into hypothyroid and euthyroid-control group (15 and 13 participants, respectively). The NO levels were determined by using of ELISA test. All subjects were referred to one experienced ultrasonographist for measurement of IMCT (mm) and SV (ms) on right femoral artery. Obtained data were analyzed by SPSS.
Results
Overall median NO levels at presentation were 37.00 ± 29.28 and differed between groups ($\chi^2 = 47.000$, $P < 0.05$). Registered median NO levels were 33.4 and 64.02 in hypothyroid and control group, respectively. An average overall IMCT and SV were 0.80 ± 0.18 mm and 0.52 ± 0.14 ms, respectively. Regarding to them, there were no statistical difference between groups ($t_{IMCT} = 1.821$; $\chi^2_{SV} = 77.500$, $P > 0.05$). NO levels neither correlated with TSH and fT_4 levels ($\rho_{TSH} = -0.256$; $\rho_{fT4} = +0.283$; $P > 0.05$), nor influenced on IMCT and SV ($\rho_{IMCT} = -0.291$; $\rho_{SV} = -0.111$; $P > 0.05$).
Conclusion
This pilot study reveals higher NO levels in euthyroid group in comparison with treatment-naïve hypothyroid females. Despite the fact that such difference is of statistical significance, small sample size and type of study are important limitations to generalize the conclusion. Additionally, there is registered no influence of NO levels on both Doppler ultrasound atherosclerosis markers. It is necessary to continue this study in regard to determine cut-off values of NO and to explain what in fact lower or higher NO levels mean in individual patient.

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P1042

Haemodialysis-induced changes in thyroid hormones and thyrotropin

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Introduction
In end-stage renal disease (ESRD), thyroid function tests are often abnormal, and their interpretation is not always straightforward. While haemodialysis (HD) procedure has well-established profound effects on the serum levels of a variety of molecules no such data have been published on thyroid hormones.
Methods
In 110 ESRD patients, total and free thyroxine (T_4 and fT_4), total and free triiodothyronine (T_3 and fT_3), reverse triiodothyronine (rT_3) and TSH levels were measured before and after a routine 4-h HD procedure using radioisotope assays.
Results
All thyroid hormones rose significantly ($P < 0.001$, Wilcoxon) during HD: T_4 by (median) 18.1% from (median) 70.9 to 91.1 nmol/l, fT_4 by 45.5% from 12.07 to 17.87 pmol/l, T_3 by 14.6% from 0.98 to 1.12 nmol/l, fT_3 by 8.7% from 3.39 to 3.61 pmol/l, and rT_3 by 15.7% from 0.265 to 0.311 nmol/l. Conversely, TSH levels after HD (median 1.60 mIU/l) were significantly lower ($P < 0.001$, Wilcoxon) than before HD (1.80 mIU/l), probably reflecting the feedback inhibition.
Conclusion
The concordant rise in all thyroid hormones, with obvious maximum in fT_4, together with a decrease in TSH, suggests an increased release of the hormones (preferentially of fT_4) from the thyroid gland. This may be due to a removal of an inhibitor during HD procedure, perhaps of an excess of iodide (Wolff-Chaikoff effect), giving further support to the presumed link between iodine excess and increased prevalence of goitre and hypothyroidism in ESRD. Other putative inhibitors among non-specific uremic toxins may also be involved, as well as metabolic acidosis correction during HD. Finally, the information about timing of blood sample (before vs after HD) may be important for proper interpretation of thyroid function tests in HD patients.

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P1043

Simultaneous occurrence of hyperthyroid and hyperglycemic emergency in a middle aged woman

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Introduction
Thyroid storm is a rare condition, reflecting an extreme physiological state within the spectrum of thyrotoxicosis. The disorder consists of the clinical manifestations of thyrotoxicosis in association with altered mental status. On the other hand, excess circulating thyroid hormones are associated with glycemic disorders, like hyperglycemia and insulinopenia. Moreover, diabetic ketoacidosis has been associated with thyroid storm in few cases. We report the case of a patient who developed hyperglycemic hyperosmolar state (HHS) and thyroid storm.
Case report
A 77-year-old woman with history of diabetes mellitus presented complaining for nausea, vomiting, dry cough and epigastric sensation for 3 days of duration. The

clinical examination revealed slight disturbance of consciousness, 40 breaths/min, 140 bpm and temperature of 37.4 °C. The chest X-ray and the urine sample were negative for infection, while the electrocardiogram showed sinus tachycardia. Laboratory studies revealed random blood glucose levels 814 mg/dl and mild metabolic acidosis, compatible with HHS. Hyperglycemia resolved rapidly after i.v. administration of normal saline and insulin, but the clinical signs remained unaffected. Additionally, the patient developed acute psychotic syndrome after 72 h. Laboratory data revealed thyrotoxicosis (TSH = 0.005 μIU/ml, FT_4 = 5.19 ng/dl), after which the patient admitted previous noncompliance to the treatment. Thyroid storm was diagnosed at this point, with a score of 70 points according to the thyroid storm score by Burch and Wartofsky. The symptoms resolved after administration of thiamazole 60 mg and atenolol 50 mg, daily.

Discussion

Thyroid storm should be diagnosed immediately using clinical criteria, in order to improve outcomes. An upper respiratory tract infection was the only apparent precipitating factor, inducing the development of both HHS and thyroid storm. However, a direct interaction between the two conditions cannot be ruled out, especially due to their simultaneous occurrence.

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P1044

Immune associations in chronic thyroiditis: December 2012

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Objectives

To investigate how the immune associations (IA) may have significance for diagnosis or therapy in thyroiditis.

Material and method

i) Patients: A. 'Classical' Hashimoto thyroiditis (hyper-ATPO-emia, HT) = 1196, B. thyroiditis with isolated hyper-ATG-emia, with normal ATPO (T-ATG) = 73, C. thyroiditis 'sero-negative' (normal ATPO and ATG, pathology diagnosis) = 8, D. idiopathic myxedema (hypothyroidism, no A,B,C) = 69; E. control = 1130. ii) Statistical analysis: χ^2 test.

Results

i) Total IA: HT = 220 (18.39%, $P \ll 0.001$); T-ATG = 20 (27.4%, $P \ll 0.001$); 'sero-negative' = 1 (12.5%, NS); idiopathic mixedema = 9 (13.04%, NS); control: 107 (9.47%). ii) Main IA: A. Graves-Basedow disease: TH: 155 (12.96%), T-TAG: 5 (6.85%); B. Vitiligo: HT = 36, $P = 0.0001$; T-ATG = 2; Control = 10. C. Dermatitis: HT = 26, $P < 0.001$. D. Immune ovaritis with precocious menopause: HT = 16, $P = 0.004$. E. Biermer anemia: HT = 13; $P < 0.001$. F. Drug allergy: HT: 24, $P < 0.001$. G. Rheumatoid arthritis: HT = 8, but in controls = 19 (NS). H. Thrombophilia: HT = 7, Control = 1 ($P = 0.04$), I. Repetitive zona zoster: HT = 7, Control = 1 ($P = 0.04$); Otosclerosis: HT = 7, Control = 2 ($P = 13$). iii) Multiple associations- very frequent (no = 100); exemples: Cerebral vasculitis with Sneddon sd, pulmonary fibrosis, cryoglobulinemia, virus C hepatitis (IFN), sicca sd; Sarcoidosis with drug allergy, scleroderma, adenomegaly, arthritis; Asthma with *postpartum* trombophilia and antiphospholipidic sd; Selective alopecia areata (no eyebrows), ferriprive anemia, miopia; Sharp disease, zona zoster, dispepsia, alopecia areata, trombocytosis.

Conclusions

i) HT and T-ATG has immune associations with increased frequency. ii) The most significant and prevalent association are: Graves-Basedow, vitiligo, Biermer anaemia, drug allergy, early menopause with immune ovaritis, thrombophilia. iii) HT, T-ATG and idiopathic mixedema are not significantly associated with other immune conditions: rheumatoid arthritis, IDDM, B/C hepatitis. iv) Multiple immune associations are common.

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P1045

Graves' disease and cardiovascular risk factors

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Objective

To evaluate the interrelationships between Graves' disease (GD) and cardiovascular risk factors.

Subjects and methods

We analyzed thyroid function tests, anti-thyroid antibodies, BMI, insulin resistance markers, namely homeostasis model assessment for insulin resistance (HOMA-IR and HOMA-B), the quantitative insulin sensitivity check index (QUICKI), hepatic insulin sensitivity index (HISI), whole-body insulin sensitivity index (WBISI), insulinogenic index (IGI) and the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein (a) (Lp[a]), homocysteine, C-reactive protein (CRP), folic acid and vitamin B12, in 106 subjects with GD (51 with overt hyperthyroidim and 55 with euthyroidism). A 75-g OGTT was performed and blood samples were obtained every 30 min for 120 min for measurements of plasma glucose, insulin and C-peptide levels. Statistical analysis was performed with Mann–Whitnney and Spearman's correlation tests. Results are expressed as mean ± S.D. and odds ratio. A two-tailed $P < 0.05$ was considered significant.

Results

94% of studied subjects were female. Mean age and BMI were similar between both groups. In hyperthyroid subjects, we found significantly higher levels of TRAb (8.3 ± 10.7 vs 2.3 ± 4.8 IU/ml, $P < 0.001$), CRP (0.84 ± 1.55 vs 0.28 ± 0.36 mg/dl, $P = 0.04$), and significantly lower WBISI values (5.01 ± 3.21 vs 6.73 ± 4.23, $P = 0.02$). In the total group, TSH levels were negatively correlated with HOMA-IR ($r = -0.22$; $P < 0.05$), IGI ($r = -0.31$; $P < 0.01$) and TRAb levels ($r = -0.46$; $P = 0.02$). FT_3 and FT_4 levels were positively correlated with HOMA-IR ($r = 0.28$, $P < 0.01$ and $r = 0.26$, $P = 0.02$, respectively) and negatively correlated with WBISI ($r = -0.23$; $P = 0.03$ and $r = -0.26$; $P = 0.02$, respectively). In the euthyroid group, TSH levels were positively correlated with WBISI ($r = 0.29$; $P < 0.05$). In the hyperthyroid group, FT_3 levels were negatively correlated with HISI ($r = -0.38$; $P = 0.02$), and TSH and TRAb were negatively correlated ($r = -0.32$; $P = 0.02$). Conclusions: The interrelationships between thyroid function, insulin resistance and CRP translate an increased cardiovascular risk in hyperthyroidism due to Graves' disease.

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P1046

Thyroid dysfunction in type 2 diabetic patients

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Introduction

While there is no consensus about population screening for hypothyroidism there is compelling evidence to support case-finding for hypothyroidism in those with autoinmune disease, such as type 1 diabetic patients. The present study has been made to estimate the prevalence of thyroid dysfunction in type 2 diabetic patients. In the British Whickham survey 9.3% of women and 1.2% of men had serum TSH values over 10 μIU/ml. The Colorado thyroid disease prevalence survey, using an upper normal TSH value of 5 μIU/ml, reported a prevalence of 8.9%.

Methods

Design: cross-sectional study. Subjects: 194 type 2 diabetic patients attending our primary health care service last year, who had determined serum free thyroxine and TSH concentrations. The upper and lower limits of the normal TSH reference range were 5.5 and 0.35 μIU/ml, respectively. Multiple clinical, demographic and laboratory parameters were measured.

Results

Mean age was 70.8 year (S.D. 11.7), and 49.5% were women. Thyroid dysfunction was present in 18 patients (9.27%; 95% CI, 5.2–13.34). Women were more likely than men to develop thyroid disorders, with a statistically significant difference ($X^2 = 4.1$; $P < 0.05$). The most common thyroid disorder was hypothyroidism (8.76%).

Conclusion

The prevalence of thyroid dysfunction in our type 2 diabetic patients was 9.27%.

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P1047

Clinical and subclinical hypothyroidism and their relation to cardiovascular risk factors

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The cardiovascular system (CVS) is one of the major targets of thyroid hormone action.
The thyroid function has a direct effect on heart rate, cardiac output and systemic vascular resistance.
The prevalence of hypothyroidism in the general population is high, particularly in females (9–15%). Overt hypothyroidism is associated with severe cardiovascular manifestations including reduced intravascular volume, increased systemic vascular resistance (SVR), and reduced contractility and cardiac oxygen consumption. Clinical hypothyroidism is associated with premature atherosclerosis and increased prevalence of coronary disease. All manifestations are potentially reversible with thyroid hormone replacement.
We have studied 132 subjects with hypothyroidism admitted in Clinic of Endocrinology Timisoara in period 2011–2012, aged 20–87 years (52.47 ± 12.23 years). They were divided by sex, age, living environment (urban, rural), value of cholesterol, heart rate, BMI, and blood pressure. Data on thyroid status, aortic atherosclerosis, and history of myocardial infarction were obtained at baseline. In the study group 89/132 patients had overt hypothyroidism and 43/132 subclinical hypothyroidism.
The study revealed an increased risk of cardiovascular abnormalities and an increased risk of atherosclerosis. Total cholesterol, triglycerides, and the systolic time intervals (increased SVR) were clearly elevated only in overt hypothyroidism. 27/89 cases with overt hypothyroidism had pericarditis.
The cardiovascular risk is associated with heart disease, but it is also influenced by the patient's age as well as the severity and duration of hypothyroidism. Close monitoring of thyroid function could be the best option for patients at high risk of progression from subclinical to overt disease.

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P1048

The usefulness of thyroid function test in patients presenting with symptomatic bradycardia/complete heart block

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Background
The causes of symptomatic bradycardia/complete are multifactorial. Bradycardia/complete heart block may be drug induced, electrolyte imbalance, ischaemic heart disease, or thyroid dysfunction. Hypothyroidism and electrolyte disturbances are reversible.
Objectives/aims
To dentify/find out whether thyroid function test where performed in patients presenting with bradycardia/CHB in keeping with good practice before insertion of permanent pace maker (PPM).
Standards
i) Thyroid function test done on admission-100%. ii) Thyroid function test done out of hours-100%.
Methods
We collected data from a list of 54 patients who needed PPM insertion from electrophysiology department/cardiology for the past 3 months.This was retrospective audit.
Results
Twenty patients (37%) had their thyroid function test prior to having PPM inserted, and 34 patients (67%) did not.
Conclusion
Hypothyroidism is reversible cause of symptomatic bradycardia/complete heartblock and thyroid function test should done prior to insertion of permanent pace maker (PPM).

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P1049

Subacute thyroiditis: unusual presentation and diagnostic troubles

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A 73 years old man came to our observation for severe dysphagia and loss of weight (10 kg in 1 month). About 30 years before he had myocardial infarction and he underwent coronary artery bypass graft. One week before the first medical evaluation, patient suspended all drugs *per os* because he could not swallow pills and food. Thyroid function test revealed a severe hyperthyroidism (FT_3 11.9 pg/ml; FT_4 40 pg/ml; TSH <0.01 μU/ml). Anti-TSH receptor and anti-TPO autoantibodies were negative while thyroid ultrasound showed an increased gland with inhomogeneous pattern, without nodules or abnormal vascularization. He did not take amiodarone. He started methimazole 30 mg/day, without any benefit. Patient then came to our evaluation about 1 month after the onset of symptoms: clinical examination showed tachycardia, enlarged and tender thyroid gland at neck palpation without relevant pain. Biochemical evaluation showed increased VES and C-reactive protein. Thyroid scintigraphy was not performed because of the interference caused by iodinate contrast medium (coronary angiography performed few days before). Neverthless subacute thyroiditis appeared strongly probable. Therefore methimazole was stopped and steroid therapy was started with i.v. methylprednisolone 40 mg for 1 week, 20 mg for 1 week and then prednisone 25 mg/day, which was tapered and continued for 30 days. Clinical symptoms, and in particular dysphagia, improved after few days of i.v. methylprednisolone while biochemical evaluation performed after 2 months showed a normalization of thyroid function test and inflammatory parameters. In conclusion, we described an unusual case of subacute thyroidits in which only dysphagia and thyrotoxicosis, without anterior neck pain, suggested an inflammatory condition. Diagnosis was made on the basis of clinical and laboratory features, because thyroid scintigraphy with RAIU, which is crucial for differential diagnosis in uncertain condition, was not possible to be performed for iodine overload.

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P1050

Simplified approach for the treatment of post-thyroidectomy hypocalcemia: a Pilot study

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Introduction
One of the notable morbidity after total thyroidectomy is hypocalcemia related symptoms. Various treatments ranging from oral to parenteral calcium have been advocated. In this context, we analysed the benefits of bolus calcium vis-à-vis infusions for symptomatic post-thyroidectomy hypocalcemia.
Patient and methods
The study was conducted in Endocrine Surgery Department in a teritiary care hospital. All the patients with benign thyroid disease, who underwent total thyroidectomy and suffered from symptomatic hypocalcemia were included. The data was prospectively analyzed seperately for Group A treated with intermittent bolus calcium and Group B treated with decrementally graduated calcium infusion. All the patients were supplemented with equal doses of oral calcium and vitamin D.
Results
The study period was 12 months (October 2011–September 2012). Group A and B included 32 and 30 cases respectively. 7/30 in Group B and 1/32 in Group A suffered from thrombophlebitis. Treatment costed mean of INR 232 in Group A and INR 665 for Group B. Both thrombophlebitis and cost factors were statistically significant. Symptomatic hypocalcemia lasted for a mean of 2.3 days in Group A vs 2.5 days in Group B, which was statistically insignificant. None of the patients in both groups suffered from permanent hypoparathyroidism.
Conclusions
Intermittent bolus calcium treatment appears to be less expensive, simpler and less morbid approach compared to continuous infusion for treating post-thyroidectomy hypocalcemia.

Keywords
Hypocalcemia; Thyroidectomy; Thrombophlebitis, vitamin D, hypoparathyroidism.
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P1051

The influence of thyroid hormones on the variability of blood pressure

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Introduction
Changes in thyroid function have significant impact on the cardiovascular system.
Methods
In a group of 37 subjects the level of thyroid hormones FT_4, FT_3, and TSH were measured. Then carried out short-term analysis for 30 min measureing variability of blood pressure with non-invasive TASK FORCE monitor. Registered data were analyzed by spectral analysis of variability. Statistical analysis were done by Pearson correlation model.
Results
The analysis of the parameters of the variability of systolic blood pressure (SBP), we confirmed that FT_4 positively correlated with parameters of spectral analysis of variability of systolic blood pressure: sBP LF; sBP LF/HF ($P<0.05$), sBP LF, sBP PSD ($P<0.001$). Discovered a negative correlation with sBP HF nu ($P<0.05$). FT_3 is positively correlated with parameters of systolic blood pressure variability: sBP LF nu, sBP LF, sBP PSD, sBP LF/HF (<0.05). TSH is positively correlated with SBP HF nu ($r=0.4379$) and HF SBP ($P<0.05$).
The analysis of correlation with thyroid hormone parameters of diastolic blood pressure variability has been found for FT_3 – negatively correlated with DBP HFnu ($P<0.5$) and positive correlation with DBP LF ($P<0.05$).
Conclusion
Our study demonstrates a significant correlation between thyroid hormones with a systolic blood pressure and less important correlation with parameters of diastolic blood pressure variability. The analysis of variability of systolic blood pressure observed a positive correlation between FT_4 and FT_3 with parts of the spectrum of low frequency (sympathetic activity) and total spectral power (PSD). FT_4 and FT_3 were in negative correlation with parts of the spectrum of high frequency (parasympathetic). TSH correlated positively with the parasympathetic nervous system.
Analysis of variability of diastolic blood pressure registered a small number of significant correlations and FT_3 – negatively correlated with DBP HFnu ($P<0.5$) and positive correlation with DBP LF ($P<0.05$).

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P1052

Serum concentrations of cholesterol and triglyceride in patients with type 2 diabetes and incident hypothyroidism

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Background
Subclinical thyroid hypofunction and elevation of serum lipid concentrations are two factors that may act synergistically in the increase of cardiovascular risk in patients with type 2 diabetes. However, the association between subclinical hypothyroidism and dyslipidemia is a matter of debate.
Objective
Our aim has been to assess whether incident hypothyroidism detected in a screening program is accompanied by elevations in the serum concentrations of cholesterol and triglyceride in a sample of patients with type 2 diabetes and no previously known dyslipidemia.
Patients and methods
From a total population of 1112 patients with type 2 diabetes who were screened for thyroid dysfunction (thyrotropin measurement) we selected a group of 325 patients with normal thyroid function and another group of 21 patients with incident hypothyroidism (20 of them with subclinical hypothyroidism). No patient had known dyslipidemia nor was taking hypolipidemic medication.
Results
Patients with incident hypothyroidism showed serum concentrations of total cholesterol (4.97 ± 0.73 mmol/l), HDL-cholesterol (1.36 ± 0.33 mmol/l), LDL-colesterol (2.99 ± 0.57) and triglyceride (1.23 (0.89–1.53) mmol/l) that did not differ from those found in euthyroid patients (4.79 ± 0.83, 1.33 ± 0.36, 2.87 ± 0.76 and 1.11 (0.81–1.43) mmol/l, respectively). When analyzing subgroups of women and patients with obesity, we could not find significant differences in lipid profile in patients with hypothyroidism and normal thyroid function. Thyrotropin concentration did not show any correlation with lipid levels in patients with incident hypothyroidism, although we found a significant correlation between thyrotropin and triglyceride in euthyroid subjects ($\rho=0.165$; $P=0.003$).
Conclusion
Our results show that, in our population, there are no significant differences in the serum concentrations of cholesterol and triglyceride between diabetic patients with normal and reduced thyroid function.

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P1053

The characteristics of thyroid pathology in patients with parathyroid adenoma

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Introduction
Thyroid and parathyroid pathology may coexist; classically, this is described in multiple endocrine neoplasia. Other nonsyndromic scenarios result in pathologies of these endocrine organs coexisting. The aim of the investigation the chracteristics of thyroid pathology in patients with parathyroid adenoma.
Subjects and method
The study population included 42 (26 patients age <60 years vs 16 patients age ≥60 years) patients with parathyroid adenoma. Clinical presentation, biochemical and radiological details were noted. Concomitant thyroid pathology by neck ultrasonography (US) in all patients.
Results
In patients with age ≥60, levels of PTH and calcium, TSH, free T_3, free T_4, autoantibody against thyroid peroxidase (TPOAb) and autoantibody against thyroglobulin (TgAb) were found to be 169.9 ± 23.7 pg/ml, 11.4 ± 1.1 mg/dl, 4.1 ± 0.9 μIU/ml, 2.4 ± 0.8 pg/ml, 0.91 ± 0.01 ng/dl, 123.7 ± 31.7 IU/ml and 57.0 ± 10.3 IU/ml, respectively. Of the 16 patients, 15 (93.7%) had thyroid nodules. Among 15 patients with thyroid nodules, 1 (6.6%) had malignant thyroid tumor. And also, 7 patients (43.7%) had hashimato thyroiditis. In patients with age <60, levels of PTH and calcium, TSH, free T_3, free T_4, autoantibody against thyroid peroxidase (TPOAb) and autoantibody against thyroglobulin (TgAb) were measured 121.9 ± 34.0 pg/ml, 12.3 ± 2.0 mg/dl, 2.9 ± 0.8 μIU/ml, 2.7 ± 0.9 pg/ml, 1.1 ± 0.1 ng/dl, 190.1 ± 42.9 IU/ml and 76.8 ± 12.1 IU/ml, respectively. Of the 26 patients, 19 (73.0%) had thyroid nodules. Among 19 patients with thyroid nodules, 2 (10.5%) had malignant thyroid tumor. And also, 12 patients (46.1%) had hashimato thyroiditis.
Conclusions
The study was demonstrated that the frequency of thyroide pathologies were high in patients with parathyroid adenoma. And also, the frequency of thyroid nodules were higher in patients with age ≥60 years than thats of patients with age <60 years.

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P1054

Learning and development of thyroid FNA in a endocrine clinic

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Background
Development of thyroid ultrasound and FNA is more frecuent to make it in a endocrine clinic, that means to make more diagnosis medical process and so to avoid more outpatients visits. But it's neccesary a specific treaning. We tried to evaluated our treaning.
Material and methods
After specific treating in radiologic department, we have compared our results in two consecutive periods. The first one in the first year after treaning developing FNAs and the second period in the next $1\frac{1}{2}$ year with FNAs. (in the second period we used Bethesda system).
Results
In the first phase we made 102 FNAs with 25 (24.5%) non diagnotic and 77 (75.5%) diagnostic, results are exhibited. In the second fhase, 220 FNAs were made and: 27 (12.27%) non diagnsotic and 193 (87.73%) diagnostic. Specific FNAs results are exhibited too. (Bethesda system: bening: 166, atipic: 14, suspicious neo folicular and Hurthle: 16, suspicius of malignant: 6, medular: 1).
Conclusions
FNAs thecnique development after period learning is possible in a endocrine clinic with satisfactory results, similar to others published series. Its useful to

patient because less visits to hospital, more efficient in your clinic activity and cheaper medical process. Bethesda system make easier comunication between clinics and pathologics.

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P1055

Does a normal thyroid ultrasound image always accompany normal thyroid functions test results?

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Introduction
Nowadays, ultrasonography (USG) is being used very commonly and indispensably for diagnosing thyroid diseases. Heterogeneous appearance of thyroid parenchyma is associated with positivity for thyroid autoantibody and hypofunctioning of thyroid gland. On the other hand, it is not determined whether thyroid parenchyma appearing totally of normal ecogenity on USG is related to presence normal thyroid function tests or otherwise. The objective of our study was evaluate the relationship between normal thyroid USG appearance and thyroid function tests with thyroid autoantibodies.
Material and methods
The study was planned prospectively and 218 patients' thyroid USG images and laboratory tests were evaluated. The participants were classified into two groups composed of the homogeneous/ normoecogenic and the heterogeneous group, according to thyroid parenchyma appearance on USG.
Findings
We acquired homogeneous images on 103 cases and heterogeneous images on 115 of the cases. TSH level, anti-Tg and anti-TPO titres and thyroid volume was found to be significantly higher in heterogeneous parenchyma group ($P<0.001$). 83 cases with normal USG images (80.5%) had normal TSH levels and 7 cases in this group had positive anti-Tg (8.4%) and 23 cases had positive anti-TPO (27.7%). Out of the heterogeneous parenchyma group composed of 115 cases; 46 patients (40%) had high TSH level and 9 of them (19.6%) had positive anti-Tg and 35 (76.1%) had positive anti-TPO. We observed no difference in between the groups in regards to fT_3 and fT_4 levels. Age and thyroid volume were determined to correlate significantly and positively ($r=0.171$, $P=0.012$).
Conclusions
The thyroid gland 'normal' appearing on USG imaging, possibly accompanies normal serum thyroid function tests and negativity for thyroid antibodies. According to our results, thyroid USG is a perfect device for predicting normal thyroid functions, which could probably avoid unnecessary future laboratory tests as well.

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P1056

The cholesterol and Lp(a) levels in Hashimato's thyroiditis

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Objective
The risk of atherosclerotic heart disease is increased in hypothyroidism. Although the reason is not clear. It is known that high level of Lp(a) is increases the risk of atherosclerotic heart diseases. The aim of this study is to investigate the relation between Lp(a) levels and thyroid hormones in the Hashimato's patients.
Methods
One hundred and fifty Hashimato's patient (50 patients diagnosed with clinic hypothryoid, 50 patients diagnosed with subclinic hypothroid and 50 patients diagnosed with euthyroid) followed at Endocrinology outpatient clinic were enrolled in this study. The control group consisted of 50 age matched healthy volunteers. In every group, thyroid function tests (FT_3, FT_4, and TSH), lipid profile and Lp(a) levels were measured.
Results
The Lp(a) level at patients with clinic, subclinic and euthyroid Hashimoto's thyroiditis is higher than the Lp(a) level of control group (P: 0.002; $P<0.01$). Total cholesterol and LDL levels were higher in all Hashimato groups than the control group. (P: 0.001; $P<0.01$). Trigliserid levels of patients in clinic hypothyroid group was higher than the control group (P: 0.006; $P<0.01$). A significant relationship was found between Lp(a) levels and LDL levels in a positive manner (P: 0.032; $P<0.05$) ($r=0.152$). There was a negative relationship between the TSH level and LP(a) level at the group of euthyroid Hashimoto's thyroiditis.
Conclusion
The total cholesterol, LDL and Lp(a) levels were higher even in euthyroid Hashimato patients than the control group. This might have been the one of the reasons of increased atherosclerotic diseases in Hashimato's thyroiditis.
Keywords
Hypothyroidism, Hashimoto's thyroiditis, Lipoprotein(a), LDL cholesterol, total cholesterol, TSH.

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P1057

Comprehensive assessment of thyroid gland structure and function in men with metabolic syndrome

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Introduction
The aim of study was to perform a comprehensive structural and functional assessment of thyroid gland in men with metabolic syndrome (MS).
Methods
Main group (MG) of 116 men aged 35–60 years with MS (by International Diabetes Federation definition) and 34 healthy controls of the same age profile underwent a comprehensive examination of thyroid gland which included physical assessment, full serum thyroid hormonal profile – total and free triiodothyronine (T_3) and thyroxine (T_4), serum TSH, and high resolution ultrasonography. Conventional criteria for hyper- and hypothyroidism by thyroid hormonal levels were used; subclinical hypothyroidism was detected by elevated TSH with normal thyroid hormone levels.
Results
Thyroid volume was higher in MG than in controls (16.6 ± 1.48 vs 10.1 ± 0.75, $P<0.05$), and overall structural abnormalities – much more frequent (in 71.6 vs 44.1%, $P<0.05$). Heterogeneous thyroid structures of all types with and without gland enlargement were seen in 19.0 and 42.2% in MG vs 8.8 and 35.3% in controls, whereas nodal changes (with size more than 1 cm) – only in MG (8.6%). Subclinical hypothyroidism was detected in 6 (5.2%) patients of MG, and in none of controls. However, in the rest of MG (cases of hypothyroidism excluded), mean TSH appeared to be lower than in controls (1.0 ± 0.19 vs 2.1 ± 0.24 mIU/l, $P<0.05$), with free T_4 being somewhat higher, albeit still within normal range (17.3 ± 0.69 vs 13.5 ± 0.63 pmol/l, $P<0.05$), and free T_3 also normal and almost the same between groups. No manifested cases of hyper- or hypothyroidism were found in any group. Thus, contrary to structure, thyroid function was mostly normal in men with MS, and there was no clear association between structural and functional variables.
Conclusion
Structural abnormalities in thyroid gland are frequent in men with MS; usually they are not accompanied by significant hormonal dysfunction, and most hormonal alterations fall within normal range.

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P1058

Empirical calcium supplements paves way for day surgery complete thyroidectomy

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Post operative hypocalcemia poses significant resistance to early discharge of total thyroidectomised patients. Our institute implemented a new protocol to give empirical calcium and vitamin D supplements since Jan 2012 to prevent symptomatic hypocalcemia and facilitate early discharge. 75 (88.24%) out of 85 eligible cases did not have significant or symptomatic hypocalcemia and can be discharged by within 1 day with only two (2.67%) patients being readmitted electively for significant hypocalcemia on follow up. While in the selected

subgroup which excludes cancer cases, concomitant primary hyperparathyroidism and retrosternal goitres to be considered for day surgery, 58 (95.08%) out of 61 were able to be discharged on first post operative day without symptoms. All those who could not be discharged reported symptoms by at most 15 h. Within this group we had not excluded reoperation and controlled Graves' disease cases, which previous series considered high risk with respect to day surgery and were excluded. Our results confirmed in well selected patient groups with routine calcium supplements and early follow up, discharging total thyroidectomised patients is feasible and safe within 24 h.

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P1059

Is TSH suppression an efficient thyroid nodular goitre therapy?

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Levothyroxine suppression therapy, i.e. keeping the TSH value below 0.3 mIU/l with the aim to reduce the total thyroidal tissue or nodule volume, is an arguable and controversial issue not only due to the effect upon nodule volume but also because of the extent of necessary suppression, the effect of incomplete suppression, long-time therapeutic effects and risk of cardiovascular problems and osteoporosis.

The incidence of nodular alterations rises with age and in patients under the age of 20 show higher malignity risk. Fine needle aspiration biopsy (FNAB) is a safe and cost-efficient method of evaluating the biological character of nodules and choosing between a conservative and surgical treatment.

In our study, we investigated nodular alterations in patients under the age of 40, evaluated malignity presence by FNAB and observed the examined nodules in treated/untreated groups for at least 1 year. The objective was to set up the criteria that can predict nodular alterations in young patients and evaluate the benefit of suppression therapy.

The 3-year (2009–2011) prospective randomized study included 78 individuals, 65 of which accomplished observation: 32 treated and 33 untreated.

The treated/untreated variances were tested using ANOVA with repetition with the following factors: group, phase, subject and interaction, which evaluates variances in time.

Our results did not confirm statistically significant reduction of nodules in patients under the age of 40 subjected to 1-year suppression therapy compared to the untreated group. The input values of all patients before treatment showed statistically significant negative correlation between the nodules size and TSH value.

The conclusion of our study is that patients suffering from thyroidal nodular alterations can be left untreated, upon FNAB evaluation, for minimum of 1 year.

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P1060

Correlation between untreated subclinical hypothyroidism, nephropathy and cardiovascular diseases in type 2 diabetic patients

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Aim

The aim of this study was to determine the relationship between subclinical hypothyroidism and prevalence of nephropathy and cardiovascular disease in type 2 diabetic patients without taking thyroid medication.

Methods

Serum thyrotropin and free thyroxine concentrations were measured in 50 type 2 diabetic subjects in DC 'Ikeda-Euromedica' and DC 'Med.al' in Tirana, from September 2009 until September 2012. In a cross-sectional study, we examined the prevalence of nephropathy. In a longitudinal study, we examined the risk of cardiovascular disease events, in the 3-year follow-up.

Results

In the cross-sectional analysis, untreated subclinical hypothyroidism was associated with a greater prevalence of diabetic nephropathy (odds ratio, 3.65 [95% CI, 1.36–6.57]) compare to euthyroid diabetics. 10% of all participants developped cardiovascular events. The risk of cardiovascular events was significantly increased in type 2 diabetics with subclinical hypothyroidism after adjustment for age, sex, HbA1c, other standard cardiovascular risk factors and medication (hazard ratio, 2.74; 95% CI, 1.04–7.37; $P=0.021$), but it became nonsignificant after additional adjustment for urinary albumin-to-creatinine ratio (hazard ratio, 2.13; 95% CI, 0.71–6.40; $P=0.217$).

Conclusions

Type 2 diabetic patients with untreated subclinical hypothyroidism are associated with an increased risk of nephropathy and cardiovascular events. This fact suggests that the higher cardiovascular events in untreated subclinical hypothyroidism with type 2 diabetes may be mediated with nephropathy.

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P1061

Relationships between antithyroperoxydase antibody levels, thyroid function and echographic patterns in chronic thyroiditis: December 2012

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Aim/objectives

To investigate relationships between thyroid function, antithyroperoxydase antibodies (ATPO), antithyroglobuline antibodies (ATG) and correlations with echographic pattern in Hashimoto thyroiditis and related diseases.

Materials and methods

Diagnosis: i) ATPO >34 μ/ml = Hashimoto thyroiditis (HT); ii) ATPO = normal with high ATG = thyroiditis with only hyper-ATG (T-ATG). Thyroid function: TSH. Echographic patterns = 10 (Peretianu, this Congress). Statistical analysis: linear correlation, χ^2 tests.

Results

i) ATPO/ATG at onset: a) ATPO: number analysis: HT-1194, T-ATG-80 and average: HT-677.7; S.D.: 1190 (!); T-ATG-11.81. b) ATG in T-ATG: 483.23; S.D.: 852 (!).

ii) Evolutional types for ATPO/ATG: a) all ATPO in HT: no. 2055, av: 740.17 IU/ml. ATPO evolution in HT: undulatorious: 150 (40.31%), decreasing: 156 (40.31%), increasing: 81 (20.93%). b) All ATG in T-ATG: no. 100, av: 415.71. ATG evolution in T-ATG: undulatorious: 4 (18.18%), decreasing: 11 (50.0%), and increasing: 7 (31.82%).

iii) TSH: a) onset HT: av: 8.83 μm/l; b) onset T-ATG: 3.63 μm/l; c) hypothyroidism HT: 41.12%; hypothyroidism T-ATG: 28.7% ($\chi^2=14.44$, $P=0.044$).

iv) Linear correlation ATPO-TSH: a) in HT: HT at onset: $r=0.17$, $P<0.001$. All HT values: $r=0.11$, $P<0.001$; b) in T-ATG: at onset: $r=-0.19$, $P>0.1$ (NS). All T-ATG values: $r=-0.17$, NS.

v) Echographic pattern 1 vs echographic pattern 8 in HT correlate with ATPO level (Table): $\chi^2>24.9$; $P<0.001$.

Table 1

ATPO levels	**Pattern 1** marked hypoechogenous pseudonodular	**Pattern 8** only slightly hypoechogenous pseudonodular	Total
0–34	21	0	21
34–100	180	12	192
100–350	324	39	363
350–999	336	25	361
1000–1999	244	10	254
2000–4999	73	1	74
>5000	37	1	38
Total	1215	88	1897

Conclusion

i) A certain correlation exists between thyroid function but only for ATPO (not ATG), only in cohorts (not <400 probes), only in HT (not T-ATG): when ATPO increases, thyroid function decreases. ii) HT vs T-ATG evolve with more hypothyroidism. iii) Pattern 8 is related low ATPO levels as compared with pattern 1. Therefore, pattern 1 suggest more inflammation than pattern 8.

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P1062

Ten ultrasonographic thyroid pattern in Hashimoto's thyroiditis: re-evaluation after 12 years

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Aim
To re-analyze the concept of echographic thyroid patterns (ETP) and clinical diagnostic in thyroidology, proposed 12 years ago.
Materials and methods
i) Between 1996 and 2012, >25 000 thyroid ultrasound, linear probes, 7.5 MHz. ii) New description – 10 ETP: pattern 0, lack of thyroid; 1, marked hypoechogenous pseudonodular; 2, hypoecogen homogenous; 3, micronodular hypoechogenous; 4, macro (>10 mm; ±micro) nodular; 5, inhomogeneous hypo/hyper-echogenous pseudonodular; 6, micronodular anechogenous; 7, hyperechogenous diffuse (normal). In 2013 we added; 8, only slightly hypoechogenous pseudonodular; 9, inhomogenously predominantly hyperechogenic with tubular anechogenic areas. iii) Patients: 1196 HT, 8 'sero-negative' thyroiditis (T S-N), 73 thyroiditis with only hyper ATG-emia (T-ATG), 70 idiopathic mixedema (IM); 72 Graves–Basedow disease (GBD) without HT; 1130 control. iv) Fiability/reliability analysis: on specificity 2012/2003 (95.68) and on sensitivity 2012/2003 (67.82).
Results
i) Number echographies/pattern/disease in Table 1.
ii) Sensitivity, specificity, and predictive positive value for the relationship pattern-diagnostic for HT vs all conditions (control + immune thyroid disease related to HT) in Table 2.
iii) Test χ^2 (54 degrees of freedom): >24.36, $P<0.001$.
iv) Reliability: specificity, 84.7% and sensitivity, 93.97%.

Table 1 Number echographies/pattern/disease.

	TH	T S-N	T-ATG	IM	GBD	Control
Pattern 0	15	0	1	7	4	9
Pattern 1	1223	1	57	17	9	84
Pattern 2	193	0	7	40	13	77
Pattern 3	74	0	3	3	3	138
Pattern 4	129	9	20	8	14	529
Pattern 5	205	0	8	1	39	39
Pattern 6	6	0	0	0	0	46
Pattern 7	74	0	5	11	13	261
Pattern 8 (new)	92	0	5	0	1	16
Pattern 9 (new)	4	0	0	0	0	2
Total	1919	10	101	87	95	1183

Table 2 Sensitivity, specificity, and predictive positive value for the relationship pattern-diagnostic for HT vs all conditions.

Patterns in HT	0	1	2	3	4	5	6	7	8 (new)	9 (new)
Sensitivity	0.78	63.73	10.06	3.86	6.72	10.68	0.31	3.86	4.79	0.21
Specificity	98.57	88.61	81.19	89.26	61.05	94.07	96.86	80.22	98.50	99.86
VPP	41.67	87.99	38.52	33.82	18.43	70.21	11.54	20.33	80.70	66.67
VPN	–	–	–	–	81.57	29.79	88.46	79.67	19.30	33.33
Accuracy	43.13	74.51	42.80	41.22	30.25	46.79	42.13	36.93	45.38	43.37

Conclusions
i) From sensitivity, specificity, and predictive positive value analysis, the classification proposed from 2003 in time (see Endocrine Abstracts 2007–2012) with only seven patterns ETP is exact and correct with ten ones, too: reliability: 84.7 and 93.97%. ii) VPP ~90 asks the diagnostic to be corroborated with antibody levels. Description 'hypoechogenous-pseudonodular' does not mean implicitly HT (could be T-ATG, too). iii) VPN >80% for pattern 4, means that, when there is a nodule over 10 mm, then HT is improbable. iv) When there are pattern 6 or 7, normality is almost sure. v) Patterns 1 and 8 suggest HT. vi) Patterns 5 and 9 suggest thyroiditis/Graves–Basedow with thyroid hyperfunction respectively euthyroidism.

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P1063

Superior vena cava syndrome due to enlarged thyroid gland

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Blood flow obstruction through superior vena cava leads to superior vena cava syndrome (VCSS). The most common cause of the VCSS is malignancies. Enlargement goitre is an unusual cause of VSSS. A patient with superior vena cava syndrome secondary to enlarged thyroid gland was presented in this case report. An 84-year-old man presented to endocrinology unit with gradually increasing neck mass, dysphagia, and shortness of breath. His physical examination revealed webbed neck with a marked thyroid swelling and multiple dilated tortuous veins with enlarged venous collaterals. An anterior posterior chest radiograph showed a widened superior mediastinum. The ultrasonography showed retrosternal goitre with heterogeneous parenchyma and nodules with cystic-necrotic degeneration. Neck computed tomography revealed heterogeneous, hypertrophic, nodular thyroid gland with multiple calcification and mediastinal extension with narrowed trachea. Thyroid function test was within normal limit. Enlargement goitres are easily recognised however, the initial presentation of superior vena cava syndrome due to substernal goitres with mediastinal extension is unusual.

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P1064

The change of character in passing of Graves' disease in St Petersburg from 1970 to 2010

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The diseases of a thyroid gland belong to the most frequent pathology which is met among people. The prevalence and structure of pathology of a thyroid gland, first of all, depends from the level of a iodine status in the population. The first time the assessment of an iodine status in St Petersburg was carried out in 1999 by the staff of faculty therapy of Saint-Petersburg State Medical University named after academician I.P Pavlov**.** The median urinary iodine concentration was 105 mkg/l. In 2010 the iodine status of St Petersburg was carried out again: the median urinary iodine concentration was significantly higher — 148 mkg/l. In this regard it was reasonable to carry a out comparison of indicators of an iodine status in St Petersburg and the features of a course of a Graves' disease before the beginning of the actions for the elimination of iodine deficiency (till 1999) and during active iodine prophylaxis.
Materials and methods
There were 310 patients with a Graves' disease, they were diagnosed during the period from 1970 to 2010.
Results
From 1980 to 2010 the number of remissions of the disease gradually decreased: till 1990 the number of remissions was 21.7% whereas from 2006 to 2011 the remissions were noted at 3.9% of patients with a Graves' disease. The group of patients with disease recurrence was analysed separately. Till 1990 among the patients with recurrence of a disease developed in 207.00±35.18 months, in the group of the diseased in 1990–1995 – in 73.00±13.95 months, in the group among the diseased in 1996–2000 – in 22.86±3.46 months, in the group 2001–2005 – in 28.59±5.19 month, and in 2006–2011 – in 7.75±1.21 months.
Conclusions
The carried-out research showed that for the last 20 years the essential change of character in the course of a Graves' disease was noted. It is possible to suppose that the revealed features, most likely are connected with the change of the iodine status of the region.
Keywords
Graves' disease, iodine deficiency, median urinary iodine concentration, iodine status.

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P1065

Subclinical autoimmune thyroid disease and cardiovascular risk factors

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Objective
To examine whether Graves' disease (GD) and autoimmune thyroiditis (AIT) are associated with insulin resistance and other cardiovascular risk factors.
Subjects and methods
We recorded thyroid function tests, BMI, insulin resistance markers comprising the homeostasis model assessment for insulin resistance (HOMA-IR and HOMA-B), the quantitative insulin sensitivity check index (QUICKI), hepatic insulin sensitivity index (HISI), whole-body insulin sensitivity index (WBISI), insulinogenic index (IGI) and the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein (a) (Lp(a)), homocysteine, CRP (C-reactive protein), folic acid and vitamin B12, in 57 patients with GD and subclinical hyperthyroidism (SCHyper), and in 83 patients with AIT and subclinical hypothyroidism (SCHypo). SCHypo was diagnosed based on raised TSH and normal free triiodothyronine (FT_3) and free thyroxin (FT_4) levels. SCHyper was diagnosed based in the context of normal FT_3 and FT_4 levels, with a TSH level suppressed below the normal range. A 75 g OGTT was performed in the morning, and blood samples were obtained every 30 min for 120 min for measurements of plasma glucose, insulin, and C-peptide. Statistical analysis was performed with Mann–Whitnney and Spearman's correlation tests. Results are expressed as mean $\pm$ S.D. A two-tailed P value <0.05 was considered significant.
Results
Ninety-five percent of studied subjects were female. In the GD group, TSH levels were negatively correlated with TRAb levels ($r=-0.28$; $P=0.04$), and FT_3 levels were positively correlated with HOMA-B ($r=40$; $P=0.06$). In this group, HOMA-IR and Lp(a) levels were positively correlated ($r=0.33$; $P=0.02$). In the AIT group, homocysteine levels were positively correlated with TSH levels ($r=0.27$; $P=0.04$), and negatively correlated with FT_3 levels ($r=-0.27$; $P=0.04$). In this group, BMI was negatively correlated with WBISI ($r=-0.37$; $P=0.004$).
Conclusion
The interrelationships between thyroid function, insulin resistance, homocysteine, Lp(a), and CRP levels translate an increased cardiovascular risk in subclinical autoimmune thyroid disease.

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P1066

Subclinical hypothyroidism, autoimmune thyroiditis, and cardiovascular risk factors

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Aim
To evaluate the relationship between autoimmune thyroiditis (AIT), subclinical hypothyroidism (SCH) and cardiovascular risk factors.
Patients and methods
We recorded thyroid function tests, BMI, insulin resistance markers comprising the homeostasis model assessment for insulin resistance (HOMA-IR and HOMA-B), the quantitative insulin sensitivity check index (QUICKI), hepatic insulin sensitivity index (HISI), whole-body insulin sensitivity index (WBISI), insulinogenic index (IGI) and the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein (a) (Lp(a)), homocysteine, CRP (C-reactive protein), folic acid and vitamin B12 in 186 subjects with AIT in euthyroid state, and in 69 subjects with AIT and SCH. A 75-g OGTT was performed in the morning and blood samples were obtained for measurements of plasma glucose, insulin, and C-peptide. Statistical analysis was performed with logistic regression and Spearman correlations. Data are expressed by mean $\pm$ S.D. Statistical significance was considered for a bilateral value of $P<0.05$.
Results
Ninety-four percent of studied subjects were female. Mean age and BMI were similar in both groups. Patients with higher levels of total cholesterol (OR = 1.008; $P=0.03$), CRP (OR = 1.684; $P=0.04$) or anti-thyroglobulin antibodies (OR = 1.002; $P=0.02$) have an increased likelihood of having SCH. In the total group, we observed a positive correlation between TSH, CPR ($r=0.13$; $P=0.04$) and HOMA-IR ($r=0.17$; $P=0.02$). We found also a positive correlation between FT_3 and HDL-cholesterol ($r=0.16$; $P<0.01$) and between FT_4 and IGI ($r=0.22$; $P<0.01$). TSH levels correlated negatively with HISI ($r=-0.17$; $P=0.02$) and WBISI ($r=-0.17$; $P=0.02$) in the total group. In the euthyroid group, there was a positive correlation between FT_3 and HDL-cholesterol ($r=0.17$; $P=0.03$) and between FT_4 and IGI ($r=0.25$; $P<0.01$). In the group with SCH, FT_3 correlated negatively with homocysteine ($r=-0.36$; $P=0.01$), and HISI ($r=-0.30$; $P=0.04$), and the levels of FT_4 correlated negatively with anti-TPO ($r=-0.28$; $P=0.02$).
Conclusions
The interrelations between thyroid function, lipid profile, CPR and insulin-resistance demonstrate an increase of cardiovascular risk in subclinical hypothyroidism due to autoimmune thyroiditis.

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P1067

Marine–lenhart syndrome

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Introduction
The Marine–Lenhart syndrome is a rare cause of hyperthyroidism that is associated with the simultaneous or sequential presence of characteristics related to Graves' disease and toxic nodular or multinodular goiter. It's validity as a disease is controversial and some authors believe that these are cases of hyperthyroidism where both etiologies coexist. The presence of hyperfunctioning nodules in Graves' disease patients ranges between 0.8 and 4.3%.
Clinical case
We present three cases of Marine–Lenhart syndrome, all in female patients, aged 40–57 years old at the time of diagnosis. All cases had positive titers of anti-thyroid antibodies and thyrotropin receptor antibodies (TRAbs). The thyroid scans showed diffuse uptake with one or more hyperfunctioning nodules, without attenuation of the surrounding areas. One of the cases represents the occurrence of hyperthyroidism after treatment of toxic nodular goiter with radioactive iodine.
Discussion
The described cases present criteria for the diagnosis of Marine–Lenhart syndrome. Graves' disease is more frequent in younger individuals, while Marine–Lenhart syndrome occurs in older ones, as occurs in Plummer's disease. As these are cases that tend to recur, total thyroidectomy is the treatment of choice.
Conclusions
Marine–Lenhart syndrome is a controversial cause of hyperthyroidism. The importance of its diagnosis resides in the preference for surgical treatment.

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P1068

Is Hashimoto's thyroiditis in children different from adults? A prospective comparative study

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Background
Hashimoto's thyroiditis (HT) is the commonest cause of spontaneous hypothyroidism. We compared clinico-investigative profile between child HT vs adult HT.
Material and methods
The study was conducted in Endocrine surgery department in a teritiary care hospital. The data was analyzed seperately for Group A: child HT (below 18 years) and Group B: adult (above 30 years).
Results
The study period was 30 months (October 2009–March 2012) with minimum follow-up of 10 months. Subjects included 95 child HT and 85 adult HT cases. 22/85 (26%) adult HT cases, required thyroidectomy for dominant nodule, pressure symptoms, etc. Cervical lymphadenopathy, diffuse goiter, painful thyroiditis rates were higher in children. Hypothyroidism in 26% of Group A compared to 4% in Group B was self-limiting
Conclusions
Significant differences in the clinical profile and natural history between child HT and adult HT seems to distinguish them as separate entities.
Keywords
Hashimoto's thyroiditis; Goiter; Hypothyroidism; Thyroidectomy.
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P1069

Evolution of thyroid function in Hashimoto thyroiditis and related disorders: December 2012

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Aim
Analyzing the evolution of thyroid function in thyroiditis and related disease during 1–17 years.
Materials and methods
i) Diagnostic: Hashimoto's thyroiditis (HT): a) antithyroperoxydase antibodies (ATPO) cut-off 34 IU/ml. b) If ATPO = normal, thyroiditis was considered if high antithyroglobuline antibodies (T-ATG). c) Idiopathic mixedema (IM): hypothyroidism, no ATPO, no ATG, and no TRAB. ii) Patients: HT: 1196; T-ATG: 73; IM: 69. Women/men: HT: 1136/60; T-ATG: 69/4; IM: 60/9 (more men: $P = 0.009$). iii) Statistical analysis: χ^2 test.
Results
i) At the diagnostic moment. a) HT: euthyroid (EUT): 534 (44.5%), hypothyroid (HOT): 498 (~41.5%), hyperthyroid (HIT): 168 (14%) – from these: 155 (~87%) associated with Graves–Basedow disease (GBD)–($TRAB^+$; more than in T-ATG). b) T-ATG: EUT: 43 (59%, more than in HT), HOT: 21 (~29%), HIT: 9 (12,33%) – from these: 5 (~55%) associated with GBD–($TRAB^+$). c) IM (by definition): HOT: 69 (100%). d) Significant difference between HT vs T-ATG: $P = 0.049$.
ii) Follow-up. a) HT: 28 (5.24%) with EUT became HOT after 0.2 (!)–8 years (av = 2.76, S.D. = 2.25). 3 (0.56%) with EUT become HIT (all GBD). 100% HOT remained HOT. 16 (9.52) with HIT become EUT after 1.5–2 years and maintain at least 5 years. 4 (2.38%) with HIT become spontaneously HOT (two with GBD). b) T-ATG: only two HIT become EUT (22%). EUT&HOT remain the same. c) IM: all remained HOT, with 1 exception (man under amiodarone who return spontaneously to EUT after withdrawal amiodarone).
Conclusions
i) Thyroiditis with only hyper-ATG could be considered different from HT. ii) HT, T-ATG, and IM presented differently as hormonal function. iii) T-TAG more than HT (but both) presented more as EUT than HOT. iv) Only 5% EUT-HT become HOT, during first 8 years. v) No EUT-HT after 8 years modified function. vi) Patients with HOT at diagnostic time, either HT, T-ATG or IM, remain HOT.
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P1070

Abstract unavailable.

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P1071

Autoimmune hypo to hyperthyroidism: a rare evolution

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Introduction
Hashimoto thyroiditis (HT; with anti-thyroid peroxidase antibodies (TPOAb)) and Graves' disease (GD; with TSH receptor antibodies (TRAbs)) are frequent autoimmune disorders responsible for thyroid dysfunction. There are two types of TRAbs, the ones that stimulate the thyroid (TSAb) causing Graves' hyperthyroidism and those that block thyrotropin action (TBAb) being occasionally responsible for hypothyroidism. Unusual patients switch from TSAb to TBAb (or vice versa) with concomitant thyroid function changes.
The progression from a HT to GD is not frequent and there are only a few cases described in the literature.
Case report
A 63-year-old woman with history of obesity and depressive syndrome was referred to our department in 2006 due to increasing weigh, with a BMI 39 kg/m^2. Neck ultrasonography (US) revealed thyroid nodular disease and the laboratory confirmed a HT (TPOAA +) with normal thyroid function.
After 1 year follow-up, the patient presented with subclinical hypothyroidism (TSH 11.0), initiating treatment with levothyroxine 50 μg/day. Fine needle aspiration biopsy (FNAB) of thyroid nodule was benign. During 4 years, the patient maintained a stable thyroid function under levothyroxine.
A five years after diagnosisof hypothyroidism, the patient presented a subclinical hyperthyroidism (TSH = 0.01). Despite levothyroxine withdrawal, she maintained hyperthyroidism complains, with weight loss, palpitations, tremor and heat intolerance, initiating treatment with methimasol and β-blockers. After scintigraphy and TRAbs titration, the diagnosis of GD was confirmed.
Discussion
The distinction between an evolution from HT to GD or from TBAb to TSAb is very difficult. Although the presence of TPOAb suggested HT diagnosis, the concomitant presence of these antibodies with TRAbs is also frequent. Nevertheless, considering the prevalence of both condition, the first option seemed most likely. Patients with thyroid function fluctuation should be closely monitored.
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P1072

The cause of haematoma on the anterior neck

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Introduction
Spontaneous cervical haematomas represent a rare condition. Few case reports have been published concerning spontaneous haematomas visible on the anterior side of a neck. These were caused by rupture of the superior thyroid artery, rupture of a thyroid nodule or the thyroid. A case of sternocleidomastoid haematoma due to haemorrhage into a nodal deposit of non-Hodgkin's lymphoma has also been described.
Case report
A 39-year-old woman previously cured of mild thyroid autoimmune hypothyroidism noticed an enlargement and pain in the thyroid area after a probable viral infection with symptoms of increased body temperature. At the same time she developed a small lump on the superior edge of her thyroid cartilage. A few days later she noted a haematoma on her anterior neck and also noticed that the lump had disappeared.
Sonographical examination confirmed thyroid enlargement with a nonspecific structural pattern and a 0.3 ml hypoechogenic spherical formation in place of the previously described lump. The haematoma was caudal from the formation. No biopsy of the formation was performed due to itś size and superficial placement of the formation.

Conclusion
The source of bleeding was not detected with certainty. We hypothesized that a rupture of a previously asymptomatic thyroglossal cyst occurred during subacute (de Quervainś) thyroiditis because of the sudden enlargement of both the thyroid and the cyst. Less probable is enlargement of the thyroid due to activation of pre-existing autoimmune thyroiditis.

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P1073

Acute respiratory failure in a rapidly-enlarging benign cervical goiter

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Benign goitres have the potential to reach massive sizes if neglected, but most have a protracted course that may or may not present with compressive symptoms. We report the case of a 57-year-old male who presented with a rapidly enlarging nodular goitre resulting in acute respiratory failure. Endotracheal intubation and emergency total thyroidectomy was done, revealing massive thyroid nodules with minimal intrathoracic extension and tracheal erosion. Despite a course and clinical findings suggestive of malignant disease, histopathology was consistent with a benign multinodular goitre. Several cases of benign goitres necessitating endotracheal intubation have been reported. Airway compromise was attributed to a significant intrathoracic component, or inciting events such as thyroid hemorrhage, pregnancy, radioiodine uptake or major surgery; none of which were present in our case. Obstructive symptoms may not correlate well with objective measures of upper airway obstruction such as radiographs or flow volume loops.

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P1074

Vitiligo: anti-thyroid peroxidase antibody

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Vitiligo is a common skin depigmenting disease, which is thought to have, at least partly, an autoimmune etiology.
Our objective was to compare the frequency of thyroid peroxidase antibody (anti-TPO) in vitiligo patients over a period of 3 years (2010–2012) in our Department.
Methods
Anti-TPO levels were assessed in order to detect any correlation with the onset, the evolution and the treatment of vitiligo. Patients with vitiligo and with known thyroid disease, history of thyroid surgery and those receiving thyroid medications were not included.
Conclusions
According to our study, high levels of anti-TPO were shown to be more common in vitiligo patients, especially in young women. As this antibody is a relatively sensitive and specific marker of autoimmune thyroid disorders and considering the fact that vitiligo usually precedes the onset of thyroid dysfunction, periodic follow-up of vitiligo patients for detecting thyroid disease, is further emphasized especially in young women with increased level of anti-TPO.
As vitiligo usually appears before the development of the thyroid disease, it may be advantageous to screen thyroid functions and antibody levels in all patients with vitiligo – this is the observation addressed to the dermatologists.
A patient diagnosed with autoimmune thyroiditis by the endocrinologist must be referred to a dermatologist in order to discover the first lesions of vitiligo, especially in the pediatric field.

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P1075

Protective antioxidative effects of caffeic acid phenethyl ester in the thyroid and the liver are similar to those caused by melatonin

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Whereas oxidative reactions occur in all tissues and organs, the thyroid gland constitutes such an organ, in which oxidative processes are indispensable for physiological functions. Thus, with additional oxidative abuse caused by several factors, increased oxidative damage to macromolecules may occur in the thyroid. In turn, numerous metabolic reactions occurring in the liver create favourable conditions for huge oxidative stress.
Melatonin is a well-known antioxidant and free radical scavenger, with protective effects against oxidative damage perfectly documented in many tissues, the thyroid and the liver included.
Caffeic acid phenethyl ester (CAPE), a component of honeybee propolis, has been suggested to be also an effective antioxidant. It is even used as a protective agent during chemotherapy and radiotherapy regimens.
The aim of the study was to evaluate the effects of CAPE on experimentally-induced oxidative damage in porcine thyroid and liver homogenates, and to compare the results with protective effects of melatonin.
Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) substrates were used to induce oxidative damage to membrane lipids (lipid peroxidation, LPO). Then, tissue homogenates were incubated in the presence of either CAPE or melatonin (0.1–500 μM) and, additionally, in the presence of Fenton reaction substrates.
Whereas CAPE decreased basal LPO in a concentration-dependent manner in both tissues, melatonin did not change the basal LPO level. When antioxidants were used together with Fenton reaction substrates, they prevented – in concentration dependent manner and to a similar extent – experimentally-induced LPO in both the thyroid and the liver.
Protective antioxidative effects of CAPE in the thyroid and the liver are similar to those caused by melatonin. CAPE constitutes a promising agent in terms of its application in experimental and, possibly, clinical studies.

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P1076

Use of scintigraphy in the current diagnosis of thyroid diseases

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Introduction
Scintigraphy had long been the only modality for thyroid gland imaging. With the development of other imaging techniques (sonography, CT, MRI), the role of scintigraphy has gradually diminished. However, it cannot be completely ignored, as it is the only technique to image not only the morphological structure, but also the functional status of the thyroid gland. In the current clinical practice, thyroid scintigraphy is performed with ^{99m}Tc pertechnetate, ^{131}I (^{123}I) at selected facilities and ^{99m}Tc MIBI in some indications.
Possible uses of scintigraphy
i) Toxic goiter, independent adenoma, multinodular goiter (Figs 1–5).
ii) Functional residues of the thyroid gland after surgery (Figs 6–9).
iii) Ectopic thyroid gland.
iv) Thyroid nodules.
^{99m}Tc pertechnetate hypofunctional (scintigraphically 'cold') and ^{99m}Tc MIBI functional (scintigrafically 'hot') nodules have a relatively significant malignant potential (Figs 10–14).
v) ^{131}I scintigraphy.
Irreplaceable in patients after thyroid gland surgery due to thyroid cancer for visualization of thyroid gland residues and distant metastases (Figs 14–16).
vi) Differential diagnosis of subacute deQuervain's thyroiditis with toxic syndrome (blockage of the accumulation of radiopharmaceuticals) and immunogenic hyperthyroidism (increased accumulation). (Figs 17–19)
vii) Parathyroid adenoma (Figs 20–22).
Conclusion
Thyroid scintigraphy has an irreplaceable role in the confirmation of thyroid residues after total thyroidectomy (carcinoma, Graves' hyperthyroidism with orbitopathy) and localization of parathyroid adenomas. It is an elegant method for differential diagnosis of the thyrotoxic syndrome in patients with subacute thyroiditis and Graves' disease. It provides a complementary technique for

detailed specification of thyroid gland nodules.
Figs 1–22 will be part of the poster.
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Thyroid cancer

P1077

Association acromegaly and thyroid cancer
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Introduction
Acromegaly is a chronic disease caused by hypersecretion of GH and IGF1, it is associated with a high risk of cancer, however the excess incidence of thyroid cancer remains controversial, we report four cases.
Patients and methods
The diagnosis of acromegaly has been placed on a dosage of GH and IGFI, the diagnostic of carcinoma is based on histological study of the surgical specimen.
Results
The sex ♀ predominant (3/4). For three patients (no. 1, no. 2, no. 4) thyroid carcinoma was the mode of revelation of acromegaly. Thyroid hormone function was normal in three quarters of cases. It was a GMN or a united nodular. The FNA made in 50% of cases was contributive in 100%. Papillary form (3/4).

Patient	Sex	Age	Goiter	TSH	FT4	TCT	Cytopunc-tion	Histological type	GH/IGF1
No. 1	♀	34	NTG	NLE	NLE	Nulle	Suspects	Papillary	>50 ng/l per NF
No. 2	♀	39	GMN	NLE	NLE	NLE	–	vesicular	0.42 mui/ml 219 ng/l
No. 3	♀	51	GMN	BASS-E	NLE	NLE	Suspects	Papillary microcar-cinoma	41. 3 mui/ml 1168 ng/l
No. 4	♂	40	NT	NLE	NLE	NLE	–	Papillary	9.5 mui/ml 217 ng/l

Conclusion
Despite the controversial results regarding the increased risk of thyroid cancer during the acromegaly, the exploring of thyroid is unavoidable and surgery must always be indicated when cytopuncture suspicious or malignant.
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P1078

Double primary carcinoma – a case of coexisting papillary thyroid carcinoma and pulmonary adenocarcinoma
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A 49 year-old Filipino male presented at the emergency room because of progressive dyspnea. He was diagnosed with papillary thyroid carcinoma by fine needle aspiration biopsy of his 7×5 cm thyroid mass 3 months prior to his current consultation. He was scheduled to undergo total thyroidectomy but he opted to postpone his surgery due to intermittent cough. On admission, a pulmonary mass with pleural effusion on the right was noted on chest radiograph. He was considered to have papillary thyroid carcinoma with pulmonary metastases and malignant effusion. Ultrasound guided biopsy of the 3×5.8×5.3 cm pulmonary mass and cytologic studies of pleural fluid, however, revealed adenocarcinoma compatible with primary lung malignancy. Chest tube thoracostomy with JP drainage was done which resulted to improvement of the dyspnea. Plan was to do pleurodesis and chemotherapy of the more aggressive malignancy, the pulmonary adenocarcinoma, then total thyroidectomy, radioactive iodine ablation and thyroxine suppression therapy of the papillary thyroid carcinoma. However, patient developed sudden dyspnea and expired from pulmonary embolism.
The coexistence of two primary carcinoma – pulmonary adenocarcinoma and papillary thyroid carcinoma is a rare event. The relationship between these two cancers is still unclear. Management should focus on the more aggressive carcinoma first. In this case, it is the pulmonary adenocarcinoma since the latter carcinoma is known for its indolent course and better prognosis than the former.
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P1079

Rac1B: a prognostic marker for papillary thyroid carcinoma?
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Introduction
Identification of molecular markers with a reliable prognostic value may greatly improve the management of patients with papillary thyroid carcinoma (PTC). Rac1b, a hyperactive splice variant of the small GTPase Rac1, was found to be overexpressed in colorectal, breast and lung cancer. It has been shown to sustain tumor cell survival in colorectal cancer and was reported to have a key role in the malignant progression of breast and lung tumors. The Rac1b tumorigenic proprieties led us to investigate whether Rac1b was expressed in PTCs.
Patients and methods
The expression of Rac1b at transcript level was analyzed in a total of 61 PTC patients (mean age 41 years; F:M ratio 43:19) by qRT-PCR. In 23 cases, tumor and the corresponding normal paired sample were compared. Rac1b expression at protein level was confirmed by western blot and immunohistochemistry in selected representative cases. Patients were divided into two groups based on longitudinal analysis and final outcome (mean follow-up 6-years): group I included patients that underwent full sustained remission after initial treatment or presented stable residual biochemical disease; group II included patients with persistence of disease after primary treatment, patients with at least one relapse and those who died from disease.
Results
Rac1b expression in thyroid tissue was clearly detected at both transcript and protein levels. Moreover, we observed that Rac1b was overexpressed in 46% of PTCs and found a striking correlation between poor clinical outcome and Rac1b overexpression ($P=0.0029$).
Conclusions
Present results document expression of Rac1b in normal thyroid cells as well as overexpression in a subset of PTCs. Whether Rac1b actively participates in thyroid tumorigenesis will require further investigation. Also, future studies are needed to validate its use as a prognosis marker.
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P1080

A single-center, open-label, phase II, proof-of-concept study with pasireotide long-acting release in patients with progressive medullary thyroid cancer: 6-month evaluation
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Introduction
Medullary thyroid cancer (MTC) is a well-differentiated neuroendocrine tumor in which somatostatin receptor (sst) expression is higher for sst_1 and sst_5 than for sst_2. This may explain why the available sst_2–selective analogues do not work in these patients and why pasireotide (SOM230), a novel, multi-receptor targeted somatostatin analogue with high-binding affinity for $sst_{1,2,3}$ and sst_5 could be effective.
Aim
To evaluate the effectiveness of pasireotide long-acting release (LAR) in MTC patients with progressive disease.
Study design
Trial enrollment started in February 2012 (study registration no. NCT01625520). Twenty patients are expected to be enrolled. At now, 14 consecutive patients with progressive metastatic or persistent postoperative MTC have been enrolled and received pasireotide LAR 60 mg/m.

Results
At 1 month evaluation, calcitonin hypersecretion was significantly decreased in seven patients (by 32–73%), stable in six and progressive in one other. Among the ten patients who were evaluable at 3 month follow-up, calcitonin was significantly decreased in six patients, stable in three and further increased in one. Five patients with bone and bowel symptoms at baseline experienced clinical response. Target tumor lesions were stable in 9/10 patients and progressive in 1/10 who had a 3- and 6-month CT scan. FDG-PET SUV_{max} was decreased by 36–50% in 2/9 patients with stable disease at CT scan.
Conclusions
This is the first experience on the use of pasireotide (SOM230) in patients with MTC. An antisecretive and antiproliferative response to pasireotide LAR has been observed in patients with progressive MTC.

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P1081

A novel multi-target pyrazolopyrimidine derivative with anti-neoplastic properties, CLM29, is active against medullary thyroid cancer *in vitro* and *in vivo*

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Introduction
CLM29, a pyrazolo[3,4-*d*]pyrimidine compound, inhibits several targets (including the RET tyrosine kinase, epidermal growth factor receptor, vascular endothelial growth factor receptor and has an anti-angiogenic effect). Recently it has been shown to inhibit proliferation and migration in primary papillary dedifferentiated thyroid cancer cells. The aim of this study is to evaluate the anti-tumor activity of CLM29 in medullary thyroid cancer (MTC).
Methods/design
The CLM29 anti-proliferative and proapoptotic effects (5, 10, 30, 50 μmol/l) were tested *in vitro* in primary MTC (P-MTC) cells obtained at surgey, in TT cells harboring (C634W) RET mutation, and in human dermal microvascular endothelial cells (HMVEC-d). TT cells were injected in CD nu/nu mice which were treated with CLM29.
Results
CLM29 (10 μmol/l, 30 μmol/l or 50 μmol/l) inhibited significantly ($P<0.001$) the proliferation of P-MTC, or TT cells. CLM29 increased the percentage of apoptotic cells in TT and P-MTC cells dose-dependently ($P<0.001$), while had no effect on migration and invasion. CLM29 inhibited significantly the proliferation, blocking extracellular regulated kinase 1 and 2 phosphorylation and inducing apoptosis in HMVEC-d. The inhibition of proliferation by CLM29 was similar in P-MTC cells with/without *RET* mutation. TT cells were injected s.c. in CD nu/nu mice and tumor masses became detectable between 20 and 30 days after xenotransplantation. CLM29 (50 mg/kg per die) inhibited significantly tumor growth and weight and the therapeutic effect was significant from the 48th day after cell implantation (18 days after the beginning of treatment). A significant reduction of Ki-67 immunostaining and of microvessel density was observed in the CLM29-treated tumors.
Conclusion
The anti-tumor activity of a 'pyrazolo[3,4-*d*]pyrimidine' compound, CLM29, has been shown in MTC *in vitro*, and *in vivo*, opening the way to a future clinical evaluation.

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P1082

Association of pre-miR-146a rs2910164 GG genotype with papillary thyroid cancer: a new case–control study on two adjacent genes on chromosome 5, pre-miR-146a and PTTG1.

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Introduction
Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy, with a steadily increasing incidence in the last few decades worldwide. Studies revealed the predisposition to PTC by the heterozygous state of rs2910164 within the precursor of microRNA146a. Interestingly, on the same chromosome, 40Kb separate the *pre-miR-146a* from the pituitary tumour transforming gene (*PTTG1*), a proto-oncogene involved in thyroid carcinomas. A genome-wide study revealed an association of the genomic region encompassing *pre-miR-146a* and *PTTG1* gene with systemic lupus erythematosus. In this study, we analyzed, with a case–control design, the genetic association between PTC and *pre-miR-146a* rs2910164 as well as *PTTG1* (rs1862391A/C and rs2910201C/T).
Methods
Two hundred and six healthy controls (30–78 of age) and 307 PTC patients (30–74 of age) were enrolled. The diagnosis of PTC was histological at surgery. Thyroid sonography was performed in controls to exclude nodules. SNP genotyping of pre-miR-146a and PTTG1 was performed by Sanger sequencing and high resolution melting. Linkage disequilibrium (LD) analysis and statistics were performed with Haplowiev 4.2 and GraphPad Prism5 software.
Results and conclusions
Pre-miR-146a rs2910164 allelic frequencies were not statistically different in patients ($C=24.3\%$) and controls ($C=28.6\%$) and the SNP was not in LD with the investigated *PTTG1* SNPs. We did not confirm a previously described association of the CG genotype with PTC. However, a significant association between the GG genotype and PTC (GG vs GC + CC odds ratio = 1.38, 95% CI 0.8–2.4) was found. The *PTTG1* SNPs (rs1862391A/C and rs2910201C/T), in perfect LD, have the same allelic frequency in patients ($A=76.7\%$) and controls ($A=76.2\%$) and are not associated with PTC. In conclusion, the study showed a new evidence of association between *pre-miR-146a* rs2910164 and PTC while *PTTG1* did not seem to be involved.

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P1083

Circulating microRNAs may help to differentiate malignant from benign thyroid nodules

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Introduction
MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs that act as negative regulators of gene expression. The miRNA expression is impaired in many types of human cancer including thyroid cancer. The tissue profile of miRNAs has been shown to be useful for differentiating benign from malignant thyroid nodules, however attainment of tissue samples requires an invasive procedure while blood sampling is minimally invasive and easy to obtain. The aim of this study was to evaluate the circulating levels of a series of miRNAs in 46 patients with nodular goiter in order to identify those that might be useful in the differential diagnosis of thyroid nodules.
Methods
Thirteen miRNAs (miR-222, miR-221, miR-146a, miR-146b, miR-21, miR-155, miR-181a miR-181c, miR-7, miR-30d, miR-126, miR-374th, miR-let7g) were extracted from serum, reverse transcribed, subjected to real-time PCR and then analyzed by the $\Delta\Delta C_t$ method. 10/13 miRNAs were evaluated post-surgically in a subset of patients undergone thyrodectomy.
Results
41/46 patients performed fine-needle aspiration cytology of the dominant nodule (20 benign, three non-diagnostic, six indeterminate, four suspicious for malignancy and eight malignant) and 28/46 patients underwent total thyroidectomy (14 benign lesions and 14 papillary thyroid cancer (PTC)). MiR-21 and -222 were higher in patients with benign histology compared to malignant. On the contrary miR-374a was significantly higher in patients with suspicious or malignant cytology and with PTC compared to those with benign disease. After thyroidectomy, the majority of miRNAs decreased while a minority of miRNAs increased or remained unchanged. Moreover miR-7 was significantly lower in patients ablated with radioiodine compared to those treated only surgically.
Conclusions
Our data, although preliminary, suggest the utility of circulating miRNAs (miR-374a showing the best diagnostic accuracy) in the differential diagnosis of thyroid nodules and the lower expression of miR-7 in patients ablated suggests its potential use as a tumor marker.

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P1084

Inhibition of proliferation in anaplastic thyroid cell lines

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Introduction
Our group has a bank of human primary thyroid cultures (BANTTIC) obtained from patients. All are individual cultures specifically genotyped and phenotyped. We believe of great interest the search for biomarkers in these cultures using differential proteomic analysis.
We have applied differential proteomics using 2D-PAGE coupled to MALDI-TOF-TOF-MS. We have compared benign pathologies (normal thyroid and Pendred's syndrome) and differential thyroid carcinomas (thyroid papillary carcinoma).
Individual protein quantities were evaluated by the Student's *t*-test within the PDQuest analysis in order to compare the two groups and identify sets of proteins that showed a statistically significant difference with a confidence level of 0.05. One of the differential spots we have found (spot-8002) is increased in carcinoma cultures (T-PC) vs benign pathologies.
Aim
To perform functional studies to investigate 8002 protein in anaplastic thyroid cell lines.
Results
8002 protein is a low molecular weight variant with two isoforms. The gene encoding spot-8002 is related with nuclear functions but also it is described to bind to cytoskeleton.
Using western blot with specific antibodies for 8002 protein, experiments that compare primary thyroid cultures from BANTTIC and commercial cell lines, have showed that 8002 protein is processed and increased in Differentiated Thyroid carcinomas (DTC) and Anaplastic Thyroid Carcinoma (ATC) in comparison with Normal Thyroid (NT).
A specific 8002 siRNA was designed. When transfected in Papillary Thyroid Carcinoma, Follicular Thyroid Carcinoma and Anaplastic Thyroid Carcinoma cell lines has blocked proliferation of Anaplastic cell lines during several days. More functional studies will be carried out to investigate this protein.

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P1085

Pre-existing autoimmune thyroid disease may influence the course of the disease in differentiated thyroid carcinoma patients

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Introduction
Differentiated thyroid carcinoma (DTC) has generally a good prognosis and is associated with prolonged survival. A minority of patients may have persistent disease and rarely aggravation during f/u. We investigated factors which may influence the persistence of disease in patients who had undergone ablation with I^{131}.
Patients and methods
903 DTC patients were referred in our center from 1968 to 2012. Five hundred and forty-one (22.4% males) underwent thyroid remnant ablation and were followed-up for 1–44 years (7.9 ± 8.4 years). Of those 344 (65.6%) showed remission during the 12 month Tg-stimulation test (Tg ≤1.0 ng/ml). Most of those who showed disease persistence (Tg >1.0 ng/ml) underwent repeated treatments (>1 surgery and/or repeat I^{131} administration), while 39.1% finally showed remission (Group 1). Those with disease persistence were further divided in three subgroups according to stimulated Tg during f/u: Group 2: low-Tg (≤5 ng/ml, no clinical aggravation (CA), $n=47$, 23.9%), Group 3: high-Tg (>5 ng/ml, no CA, $n=50$, 25.4%) and Group 4: CA-group ($n=23$, 11.7%).
Results
Patients with 12 month remission of disease had more frequently previous history of autoimmunity ($P=0.008$). Men had less frequently remission at f/u and more frequently CA compared to women (26.3 vs 44.3%, 19.3 vs 8.6%, $P=0.004$). Group 1 had more frequently positive thyroid auto antibodies at diagnosis (Group 1: 72.7%, Group 2: 11.1%, Group 3: 12.5%, Group 4: 0%, $P=0.003$), and microDTCs (<1.0 cm), (28.6, 11.8, 12.5, 0%, $P=0.002$), less frequently lymph-node involvement (35.4, 62.8, 57.1, 84.2%, $P<0.001$), soft-tissue involvement (12.1, 8.6, 29.8, 52.9%, $P=0.002$), and distant metastases at diagnosis (0, 10, 8, 60%, $P=0.003$). More aggressive histological type was more frequently observed in Group 4 ($P<0.001$). No differences in multifocality, family DTC history and type of first surgery were observed between groups. Age at diagnosis, tumor size and Tg levels at the time of the first ablation were higher in groups 3 and 4 ($P<0.04$, Mann–Whitney).
Conclusion
In addition to classical favourable prognostic factors such as smaller size and lack of invasive features, pre-existing autoimmune thyroid disease may be associated with early remission in patients with DTC.

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P1086

Evaluation of treatment approaches to the sporadic and hereditary medullary thyroid carcinoma in Turkey

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Introduction
RET mutation analysis has a critical importance for determining clinical approach to hereditary medullary thyroid carcinoma (MTC). The current guidelines recommends to determine the timing of prophylactic thyroidectomy depending on the risk stratification, mainly decided by the type of the mutation.
Methods
Society of Endocrinology and Metabolism of Turkey (SEMT) sponsored ret genetic screening between July 2008 and January 2012 in 513 patients. Application forms of 513 patients whose blood samples were sent by their doctors to SEMT were assessed. We aimed to evaluate 319 eligible patients who had sporadic, familial MTC, MEN 2 or mutation carriers from known families. Physicians were asked to give information about, surgical history, latest calcitonin levels, survival of the patients and also further genetic screening and prophylactic thyroidectomies among family members. An evaluation form had been prepared for each patient and sent to their doctors all around the country. Twenty-five centers responded by filling in the forms of 192 patients.
Results
Mean follow-up period was 40 months. At the time of diagnosis 15 (13%) patients had stage 4 disease and 13 (86.6%) of these cases were sporadic. In their last control 80 patients (41.9%) were in remission, 21 patients (11%) had locoregional disease and 29 (15.2%) had distant metastases. Seven sporadic and one MEN2A

patient were lost due to metastases. Sorafenib was the most preferred chemotherapeutic drug in metastatic patients ($n=14$). Prophylactic thyroidectomy was performed only in ten patients and mean prophylactic thyroidectomy age was 35. There were 14 patients who had not yet been operated although RET mutation was detected.

Discussion

MTC is a relatively rare thyroid cancer. Although genetic testing is available with the courtesy of SEMT. Unfortunately the numbers of the prophylactic thyroidectomies is still insufficient and the timing is late.

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P1087

Sentinel lymph node biopsy in thyroid carcinoma and decision for selective modified radical neck dissection

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Background

The accuracy of sentinel lymph node biopsy (SLNB) in decisions for surgical management of lymph nodes in thyroid carcinoma (TC) was demonstrated in a few previous studies. SLNB was started in good selected cases of medullary TC (MTC) and it is a promising method. Its application to avoid prophylactic neck dissection

Aim

To determine whether SLNB of first draining node/s in jugulo-carotid chain is accurate technique to select patients with true positive LN for selective modified radical neck dissection (MRND).

Patients and methods

We have performed SLNB in 172 patients with papillary TC and 12 cases with MTC. Before mobilization of the thyroid gland, 0.2 ml of 1% solution of methylene blue dye was injected peritumorally. After 10 min the dissection was continued around omohyoid muscle, towards the internal jugular vein and carotid artery until blue stained LN were found and sent for frozen-section examination. An extended dissection of level III/IV was done consecutively. All LN were examined by frozen section and conventional (HE) histopathology examination. If positive, MRND was performed after total thyroidectomy and routine dissection of central neck compartment.

Results

Identification rate of SLN was 94.5%. Specificity and sensitivity of the method were 99.3 and 84.4% respectively. Negative and positive predictive values were 96.5 and 96.4%. Overall accuracy of the method was 96.5%.

Conclusions

According to previous data, status of lower jugulo-carotid LN significantly predicts the status in upper two thirds. Our results imply that SLNB in the jugulo-carotid chain using methylene blue dye mapping, is feasible and accurate method for estimating LN status in the lateral neck compartment. The method may support a decision to perform selective MRND in patients with TC. Also it is promising method for MTC in clinical N0 stage.

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P1088

Pre-miR146a expression profiling of follicular thyroid carcinoma

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Introduction

Follicular thyroid carcinomas (FTC) are the second most common type of thyroid malignancy. Micro-RNAs (miRNAs) are a new class of small, noncoding RNAs whose expression is deregulated in many types of human cancers and may lend novel clues to FTC genesis. Pre-miR146a represents one of the most up-regulated miRNAs in papillary thyroid carcinomas (PTC) and a SNP (rs2910164), identified in pre-miR146a, contributes to genetic predisposition to PTC, but data on FTC are still lacking. The objective of the study was to evaluate the expression of pre-miR146a in FTC both in neoplstic and non-neoplastic tissues that in relation to the rs2910164 SNP of pre-miR146a.

Design

Pre-miR146a expression levels were detected in 35 male and female patients with FTC, aged 53 ± 18. RNA was extracted from surgically removed thyroid neoplastic and non-neoplastic FFPE samples. Total RNA containing miRNAs was used for stem-loop RT reactions. A standard TaqMan PCR kit protocol was implemented for real-time PCR. Reactions were performed on the CFX96 Real-Time System (Bio-Rad) with U6 RNA as an endogenous loading control. The pre-miR146a common G/C polymorphism, designated rs2910164, was genotyped by sequencing. Wilcoxon signed-rank test and Friedman test were used for statistical analysis.

Results

The expression of pre-miR146a is significantly down regulated in tumor compared to non-neoplastic tissues in patients with FTC ($P=0.043$). rs2910164 genotype is related neither to the level of expression of pre-miR146a nor to the type of tissue analyzed. Finally, no correlations between the expression of pre-miR146a and the genotype of the SNP in the transition from non-neoplastic to neoplastic tissue in patients with FTC were found.

Conclusions

The expression of pre-miR146a is related to FTC but not to the rs2910164 SNP.

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P1089

^{68}Ga-DOTA-NOC PET/CT role in the follow-up of patients with medullary thyroid carcinoma

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Introduction

About 50% of patients with medullary thyroid carcinoma (MTC) have persistent or recurrent disease after surgery.

For a calcitonin (ct) value greater than 150 pg/ml, it is suggested by international guidelines performing additional imaging exams (AIE) for evaluating the presence of distant metastasis. It is often difficult to assess the presence of nodal disease and secondary liver lesions (often miliary) by 'morphological' imaging (MI).

Objectives

To evaluate the role of ^{68}Ga-DOTANOC PET/CT in the detection of residual disease or recurrence/metastatic lesions in patients with biochemical evidence of recurrence or persistence of CMT.

Methods

Retrospective study of patients diagnosed with MTC who underwent surgery and performed ^{68}GaDOTANOC PET/CT at our institution between August 2010 and March 2012.

Results

Twenty-three patients, 16 (69.6%) females, aged between 26 and 78 years (median 54). ^{68}Ga-DOTANOC PET/CT was performed from 4 months to 21 years, after the initial diagnosis of CMT (median 5 years). The main reasons for the exam's request were high ct and/or no evidence of disease in conventional AIE and to evaluate the extent of the disease. In 65.2% of cases, ct value was >150 pg/ml. The exam revealed lesions in 12 cases (52.2%) and all of these had ct levels >150 pg/dl. Among 12 patients with previous negative MI, ^{68}Ga-DOTANOC PET/CT showed evidence of disease in five cases (42%). It provided additional information to MI in four cases.

Conclusions

In our series, ^{68}Ga-DOTANOC PET/CT provided additional information in about 39% of cases. These results support the important role that ^{68}Ga-DOTANOC PET/CT may have in the follow-up of CMT patients, particularly those with significantly high ct values. It could even select potential candidates for PRRT therapy.

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P1090

Distribution of ret proto-oncogene mutations among Turkish familial medullary thyroid cancer/multiple endocrine neoplasia 2 patients

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Introduction
TURK-MEN study was carried out to evaluate the mutational analysis of so called sporadic MTC patients between 1994 and 2005. Our aim was to evaluate the ret genetic screening results, distribution of mutations among so called sporadic and hereditary MTC patients in Turkey between 2008 and 2012.
Methods
Society of Endocrinology and Metabolism of Turkey (SEMT) sponsored ret genetic screening between July 2008 and January 2012 in 513 patients. We excluded family members of known patients who do not have ret mutation and patients with pheochromocytoma without mutation. We invited physicians of 319 eligible patients who had sporadic, familial MTC, MEN 2 or mutation carriers from known families and forms of 192 patients were attained. Analysis of exon 10, 11, 13, 14, 15 and 16 was performed.
Results
Between 2008 and 2012, among 513 patients whose blood samples were sent for evaluation, ret genetic mutation was detected in 71 patients (13.8%). Cys634Arg mutation was the most prevalent mutation. Cys634Arg mutation was detected in 31 patients (43.6%), Val804Met was detected in 18 patients (25.3%) and Cys634Tyr mutation was detected in six patients (8.4%). Other rare mutations were Tyr791Phe, Cys618Ser, Met918Thr, Cys634Gly, Y790Phe, Tyr790Phe, Leu790Phe, Ser891Ala. The number of mutation carriers among 154 apparently sporadic MTC patients was 13 (8.4%).
Conclusion
This is the largest mutation analysis ever done in Turkey. This trial shows that Val804 mutation is also an important mutation with 25.3% frequency in Turkish population besides Cys634 mutations.

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P1091

Treating refractory thyroid cancer in the era of multitarget tyrosine kinase inhibitors

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Introduction
Tyrosine kinase inhibitors (sorafenib and sunitinib) have been used in treating refractory cases of thyroid cancer. The aim of our present study was to assess the efficacy of these agents in patients with refractory and progressive thyroid cancer, regarding patients' quality of life, adverse events and response rates
Patients and methods
We retrospectively analyzed data of adult patients with differentiated and medullary thyroid cancer (DTC and MTC) treated with either Sorafenib or Sunitinib. Disease progression was assessed according to RECIST criteria. Adverse events were documented and graded with the use of the NCI Common Terminology Criteria for Adverse Events. Patients received treatment until disease progression, serious-grade 4-adverse events or until unwilling to continue treatment due to adverse events.
Results
From April 2009 to October 2011 a total of 16 patients (nine males and seven females) were included. Six patients with MTC and ten patients with DTC were placed on tyrosine kinase inhibitors. The median duration of treatment was four circles (range 0.5–16). Discontinuation of treatment was noted in 14 out of 16 (87.5%) patients, who were unwilling to continue treatment due to side effects, and reported worsening of quality of life. Though most adverse events were grade 1 and 2, two patients experienced grade 4 serious adverse events. Life threatening were: stage 4 heart failure in one patient, reversible with treatment withdrawal, and stage 4 nose bleeding noted in another. More than 50% of patients reported fatique, diarrhea, stomatitis and dermatologic disorders. Main laboratory abnormalities were anemia, neutropenia and hypertriglyceridemia. Dose reduction was required in five out of 16 patients. Concerning overall response, two patients showed partial response, nine stable and five progressive disease. Metastatic sites favouring response were lungs and liver.
Conclusions
Treatment with tyrosine kinase inhibitors should clearly balance benefits and risks of worsening patients good quality of life.

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P1092

Mutations and somatic changes in the genotype of rs2910164 in pre-miR146a are frequent in follicular thyroid carcinoma

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Introduction
The mechanism underlying the development of follicular thyroid carcinoma (FTC), the second commonest thyroid tumor, is still unknown. An interesting hypothesis is that changes in the expression of multiple regulatory RNA (miR) genes may be a major mechanism in thyroid carcinogenesis. For example, several studies suggested that SNP rs2910164 in the pre-miR146a, a precursor of miR146a, might be correlated to papillary carcinoma. The aim of this study was the evaluation of the role of SNP rs2910164 in FTC tumorigenesis. Here for the first time we analysed the genotype frequencies of rs2910164 in both genomic and somatic DNA of patients affected by FTC.
Materials and methods
The region of pre-miR146a containing SNP rs2910164 was sequenced in both genomic and somatic DNA of patients affected by FTC ($n=39$). Somatic DNA was extracted from formalin-fixed-paraffin-embedded tissue, while genomic DNA was from peripheral blood. We compared the SNP distribution between patients' genomic and somatic DNA (both unaffected and tumor tissue). In addition, patients' genomic DNA was compared to 208 controls with negative thyroid sonography. SNP distribution was correlated to the clinical data.
Results
SNP rs2910164 undergoes mutations in the transition from genomic to somatic DNA in 37% of cases and from unaffected to tumor tissue in 31% of cases resulting in an increase of allele G frequency in tumor tissue ($P<0,05$, χ^2 test), in which CC genotype was completely absent. The SNP distribution in the patients genomic DNA was the same as in negative controls ($P=0.9106$; χ^2 test). No correlation between this SNP and clinical features was found.
Conclusion
Our data suggest that somatic GG and GC genotypes are associated with FTC, while CC homozygous state might have a protective role. This could result in modifications of target genes, the expression of which is affected by the SNP status.

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P1093

Interobserver agreement of thyroid imaging reporting and data system and realtime elastography for the assessment of thyroid nodules

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Introduction
Work-up of thyroid nodules remains challenging. Thyroid imaging reporting and data system (TIRADS) has been developed to improve patient management and cost-effectiveness by avoiding unnecessary fine needle aspiration biopsy (FNAB) in patients with thyroid nodules. However, since its publication in JCEM (2009) its clinical use is still very limited and its practicability in clinical practice is questioned. Realtime-elastography (RTE) enables the determination of tissue elasticity and has shown promising results for the differentiation of thyroid nodules.
Methods
The aim of the present study was to evaluate the interobserver agreement of TIRADS and RTE. Three blinded observers independently scored stored images of TIRADS and RTE in 114 nodules of 114 patients. In addition, the diagnostic performance of TIRADS and RTE for the diagnosis of malignant thyroid nodules was calculated. Cytology and/or histology was available for all benign ($n=99$) and histology for all malignant nodules ($n=15$).
Results
The interobserver agreement between the three physicians was only weak for TIRADS categories 2–5 (Cohen's $\kappa=0.27$, $P=0.000001$) and TIRADS categories 2/3 vs 4/5 (ck$=0.25$, $P=0.0020$). The interobserver agreement was substantial for RTE scores 1–4 (ck$=0.66$, $P<0.000001$) and very good for RTE scores 1/2 vs 3/4 (ck$=0.81$, $P<0.000001$). 92–100% of patients with TIRADS 2 had benign lesions, while 28–42% with TIRADS 5 had malignant cytology/histology. The negative predictive value (NPV) was 92–100% for TIRADS using TIRADS categories 4 and 5 and 96–98% for RTE using score 3 and 4 for the diagnosis of malignancy, respectively. However, only 11–42% of nodules were in TIRADS-categories 2 and 3, as compared to 58–60% with RTE-score 1 and 2.
Conclusions
Interobserver agreement of TIRADS is only fair. TIRADS and RTE have high NPV for excluding malignancy in the diagnostic work-up of thyroid nodules. However, only 1/3 of FNABs could be avoided using TIRADS as compared to 60% using RTE. Prospective multicenter studies are needed to further evaluate the clinical utility of both methods.

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P1094

Medullary thyroid carcinoma – a narrow surgical window

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Introduction
Occurring sporadically or as part of a familial syndrome, medullary thyroid carcinoma (MTC) is insidious in onset, aggressive in behavior and invariably a challenge to the clinician and surgeon.
Objective
To define the relationship between biochemical parameters, disease progression, and treatment outcome in patients with MTC.
Patient and methods
We studied 35 patients (11 men, 24 women) aged 50.9 ± 14.8 years, admitted to the National Institute of Endocrinology between 2004 and 2012 and diagnosed with MTC: six with MEN2A syndrome, three with familial MTC with documented RET mutation and 26 patients with sporadic MTC. Most patients presented with a thyroid lump or for screening if from a MEN2A kindred, seven with chronic diarrhea and 2-paraneoplastic Cushing syndrome.
Results
Calcitonin at diagnosis was 1586 ± 1924 pg/ml (25 of 35 patients with available data); 36% were >2000 pg/ml, the upper limit of the assay. TNM classification at diagnosis shows 56.67% of patients in stage IV, 33.33% stage III, 3.33% stage II, and 6.67% stage I – highlighting the aggressiveness. The onset of symptoms was 22.5 ± 29.8 months before diagnosis, delayed in two cases by false negative FNAB. Most patients underwent total thyroidectomy with (60%) or without neck dissection (11.4%), in 22.9% partial thyroidectomy was performed and in two patients (5.7%) oncologic evaluation without surgery was recommended because of wide metastatic spread; 39.4% required multiple surgeries. Calcitonin decreased to 764 ± 2103 pg/ml at 3–6 months postop while the carcinoembrionic antigen decreased from 147.3 ± 283.9 to 5.35 ± 7.04 ng/ml. Only eight patients showed biochemical remission at 3–6 months postop: two patients were TNM stage I at diagnosis, six were stage III. In patients with persistent disease, adjuvant therapies were tried: chemotherapy, external radiotherapy, internal radiotherapy, somatostatin analogues, radioiodine and IFN.
Conclusion
Despite the potential for cure of MTC with timely intervention, optimal results are still achieved in a minority of cases, mostly due to the delayed diagnosis. After the 'surgical' window has passed, even a sustained multidisciplinary effort leads to poor results.

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P1095

Prognostic value of postoperative thyroglobulin levels for papillary thyroid carcinoma

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Background
Patients with papillary thyroid carcinoma may have repeated recurrence. This study evaluated the prognostic value of postoperative thyroglobulin levels.
Method
From 1998 to 2009, patients with papillary thyroid carcinoma were recruited by medical chart review. The pathology report, postoperative thyroglobulin levels, radioactive iodine (RAI) ablation and scan, and re-operation were abstracted.
Results
A total of 453 patients (125 male and 328 female, mean age 47.4 ± 15.9 years) with papillary thyroid carcinoma were recruited. The postoperative staging I, II, III, IV was 57.6, 6.4, 14.8, 21.2%, respectively. The mean postoperative thyroglobulin level was 51.7 ± 251.8 ng/ml. The thyroglobulin level was stratified to I (<2), II (between 2 and 10), III (between 10 and 30), IV (between 30 and 100), V (more than 100). The association between stage and thyroglobulin level was not significant (χ^2, $P=0.0621$). Postoperative thyroglobulin level was not significantly associated with the results of RAI ablation scan. During the follow-up period (mean 3.4 ± 2.6 years), 102 patients (22.5%) received repeated operation or RAI treatment for recurrent tumor or lymphnode metastasis. The recurrence rate in thyroglubin level I, II, III, IV, V was 12.7, 20, 18, 27.5, 41.7%, respectively. The predictive effect of postoperative thyroglobulin levels for tumor recurrence was not statistically significant (logistic regression, $P=0.5985$).
Conclusions
For patients with papillary thyroid carcinoma, post-operative thyroglobulin level was not associated with recurrence rate. Careful long-term follow-up was necessary even for patients with low thyroglobulin level.

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P1096

Radioactive iodine therapy in papillary thyroid carcinomas staged as T1

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Introduction
^{131}I therapy in patients with papillary thyroid carcinomas (PTC) ≤2 cm and without extrathyroidal extension (T1) depends on multifactorial analysis: age, multifocality, histological criteria, lymph node or systemic metastasis. The study purposes were analyze PTC-T1 and compare the groups treated only with surgery vs combined therapy (surgery and ^{131}I).
Methods
Retrospective analysis of clinical files of PTC-T1 patients diagnosed between 2002 and 2006, and followed in Endocrinology Department of Portuguese Institute of Oncology, Lisbon. Patients were identified through South Regional Cancer Registry.
Results
A 178 PTC-T1 were identified. The mean age at diagnosis was 47.6 years (9–79). 85.9% were female (F/M$=6.1:1$). Mean tumour diameter was 1.2 cm (50% were T1a, meaning ≤1 cm). 4.5% had aggressive histological variant; 32.6% were

multifocal; 5.1% had angioinvasion; 16.9% and 2.2% had lymph node and lung metastasis, respectively. 5.6% had positive surgical margins. Mean follow-up time was 71 months (±23).
From 178 PTC-T1, 109 patients were submitted to ^{131}I therapy (72.2%). 65.1% were T1b; 55% had more than 45 years, 44% were multifocal; 7.4% had aggressive histological variant; 8.3% had angioinvasion; 26.6% had neck lymph node metastasis and 3.7% had lung metastasis. 87.1% are considered in complete remission. Hypothyroidism pre-^{131}I was achieved by thyroid hormone withdrawal in 78.9% cases and by rhTSH stimulation in the remaining; TSH level at radioiodine therapy was considered 'insufficient' (<30 μUI/ml) in 19.1% (all in the former group). One hundred and thirty-two therapies were administered (activities ranging between 50 and 155 mCi).
From the 69 cases treated only with surgery, none presented risk histological criteria, namely aggressive histological variant, angioinvasion, nodal or systemic metastasis. All are considered in complete remission.
Conclusion
Generally, PTC-T1 are associated with good prognosis. In the absence of metastasis and/or aggressive histological criteria, the benefit of ^{131}I therapy is doubtful. A trend to reduce ^{131}I therapies was noticed in this study.

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P1097

The role of radioiodine SPECT/CT in differentiated thyroid cancer

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Introduction
Beside remnant ablation and treatment of known metastases, radioiodine treatment of differentiated thyroid cancer is suitable for the discovery of unknown or suspected metastatic disease. In this study, the clinical utility of SPECT/CT, a hybrid imaging method was evaluated after radioiodine therapy.
Patients and methods
Between July 2007 and March 2012, 260 investigations were performed at 188 patients. Male:female ratio was 47:141. Every patient was evaluated by whole body scanning (5 cm/min), planar (100K) and SPECT/CT imaging (SPECT: two detectors, 50 s/frame, 64 frame; CT: low dose, 16-slice helical CT, 120 KeV, 50 mAs) 6 days after 1100–3700 MBq radioiodine treatment.
Results
Only remnant thyroid tissue was detected in 122 patients (47%). Absence of any radioiodine uptake plus a negative CT was found during 16 investigations (all were after repeated treatment). Pathological isotope accumulation was diagnosed with positive CT result in 96 cases (37%). Radioiodine uptake with suspected clinical significance without CT abnormality was found in 22 cases (8%). Radioiodine negative metastases were discovered during 21 investigations (8%). Lymph node, lung, bone and other metastases were diagnosed in 79, 41, 19 and 12 cases, respectively. Nonspecific radioiodine uptake was found during 23 investigations caused by gluteal hematoma, kidney cyst, inflamed ulcer of the lower extremity, foreign body in the neck, gingivitis, isotope contamination of the skin and body hair, etc. As a consequence of a positive SPECT/CT imaging, 19 patients were operated after radioiodine treatment, mostly by gamma-probe guided surgery.
Conclusion
The SPECT/CT hybrid imaging method after radioiodine treatment proved to be useful in the discovery and localization of metastatic disease, exclusion of artifacts, therapeutic setting and indication of surgery.

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P1098

Examination of CYP24A1 and 'three-genes' (SFN, MRC2, HMGA2) expressions in different pathological subgroups of human papillary thyroid cancer

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Background
The 'three-genes' (SFN, MRC2, HMGA2) might have direct role in various cancer progression and malignant thyroid conditions. We previously reported an increased expression of 1,25-D_3 neutralizing enzyme, (24-hydroxylase – CYP24A1) as a possible novel marker gene in thyroid carcinoma. In this study, we examined the transcription activity of CYP24A1 and 'three-genes' in four functional subgroups of papillary thyroid tumor (PTC).
Methods
The gene expression analyses of more than 50 thyroid carcinoma/normal tissue pairs were carried out. Subgroups were defined by the following criteria: i) presence of somatic oncogene mutation and/or rearrangement; ii) conventional PTC or other PTC variants; iii) other thyroid disease present beside PTC; iv) lymphnode metastasis and/or vascular invasion are detected.
Results
We demonstrated that 'three-genes' expression was increased in 79.6% of PTC tissues that increased to 87.1% if CYP24A1 expression was added as a fourth gene. In group 1, higher rate of elevated 'three-genes' activities were seen if tumor tissues carried a somatic oncogene mutation. However, there is no difference in distribution of high-level CYP24A1 tumors between carriers and non-carriers. In group 2, we showed increased CYP24A1 expression ratio in conventional PTC samples vs PTC variants. No significant alteration was seen in proportion of enhanced 'three-genes' expression. In group 3, we observed greater ratio of CYP24A1 overexpressed tumors in PTC samples without any other thyroid disease. In group 4, higher percentage of tumor samples showed increased expression of all the examined genes if lymphnode metastasis and/or vascular invasion were detected.
Conclusions
Our results suggest that 'three-genes' model completed with CYP24A1 might increase the efficiency of molecular diagnosis of papillary thyroid cancer. Also, we have demonstrated that certain pathological states within PTC are associated with altered gene expression.

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P1099

The prevalence, the tumorigenic role and the functional implications of rare BRAF alterations in a cohort of Italian patients with thyroid carcinomas

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Background
Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid gland, accounting for 74–80% of all thyroid cancers. The T1799T>A transversion is an activating mutation of the BRAF oncogene that is common in conventional PTC and specific to it.
Aims
To study the prevalence, tumorigenic role and biomolecular implications of rare *BRAF* variants in a large cohort of patients.
Study design
A 1641 fine-needle aspiration biopsy samples were collected and subjected to *BRAF* mutation analysis: 494 were PTC. The rare genetic variants found were also analyzed by western blot to investigate their susceptibility in modulating fundamental signaling pathways, by immunofluorescence and by means of *in silico* analysis to evaluate their molecular role in large-scale exploration of conformational spaces.
Results
BRAF mutations were found in 271/494 (54.9%) of PTC. They were classic (c.1799T>A) mutation (in 97%) and rare genetic variants (in 3%). A total of nine infrequent alterations were detected: c.1795_1797dupACA (p.T599dup) found in two patients (one with the follicular variant and the other with classic PTC); c.1801A>G (p.K601E) found in three patients (one with poorly differentiated follicular carcinoma and two with the follicular variant of PTC); c.1799_1801delTGA (p.V600_K601>E) found in three patients (one with

hobnail, one with tall cell variant and the last not yet operated): and c.1799_1814>A (p.V600_S605>D) in one patient (classic PTC variant).
Conclusions
This study delineated the prevalence, tumorigenic role and functional implications of rare *BRAF* alterations of thyroid carcinoma.
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P1100

Thyroglobulin in fine-needle aspiration wash-out diagnostic performance: a meta-analysis
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Introduction
Differentiated thyroid cancer (DTC) has an excellent prognosis. However, DTC frequently metastasizes to cervical lymph nodes (CLN). In case of suspicious ultrasonography findings, the fine-needle aspiration (FNA) is usually required to confirm or exclude metastasis. The combination of FNA-cytology and thyroglobulin (Tg) measurements in the needle washout has been reported to increase the sensitivity of FNA in identifying lymph node metastases from DTC, particularly in the case of very small CLN. This assay is recommended by the revised American Thyroid Association guidelines for the follow-up of patients with DTC.
Methods
We have selected, through electronic databases, 28 original studies, published from 1992 through 2012. Each study deals with a small number of patients and is likely affected by selection bias. Pooled sensitivity, specificity, likelihood ratios (LR) and diagnostic odds ratio (dOR) were calculated.
Results
Including in the analysis all the studies with complete data (17 studies), the pooled sensitivity is 95.8% (95% CI 94.3–97%), specificity 91.5% (95% CI 89.1–93.5%), positive LR 15.33 (95% CI 6.97–33.70), and negative LR 0.06 (95% CI 0.04–0.11). However, there is a significant heterogeneity between studies, not due to threshold effect (Spearman correlation coefficient -0.146; $P=0.52$). A meta-regression analysis demonstrated that the presence or absence of thyroid gland (evaluation before thyroidectomy or during after-surgery follow-up) is an important heterogeneity factor (rdOR 4.57; 95% CI 1.34–15.61; $P=0.02$). Including only studies reporting data from patients during follow-up (ten studies), the pooled sensitivity is 98.7% (95% CI 96.8–99.7%), specificity 97.9% (95% CI 95.2–99.3%), positive LR 17.47 (95% CI 7.81–39.10), and negative LR 0.04 (95% CI 0.02–0.09).
Conclusion
FNA-Tg measurement is a very accurate tool to detect CLN metastases from DTC. However, a better standardization of criteria for patient selection, analytical methods and threshold levels is required to provide useful data and to improve management of DTC patients.
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P1101

Thyroglobulin levels in the washout of lymph node fine-needle aspirate on patients with previous history of differentiated thyroid cancer
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Introduction
Our aim was to determine the comparable value of thyroglobulin in the washout of lymph node fine-needle aspirate (FNATg) and fine-needle aspiration biopsy (FNAB).
Materials and methods
We included 29 patients (37 FNAB) with history of differentiated thyroid cancer who underwent total thyroidectomy and were found to have suspicious cervical lymph nodes during follow-up. The referred population was evaluated on what concerns to gender, age, serum thyroglobulin, thyroglobulin antibodies, FNATg, FNAB and lymph node echographic features.
Results
Cohort with 69% females; mean age of 45.9 ± 15.1 years. Considering the serum thyroglobulin levels, 72% patients had disease's persistence.
FNATg levels were undetectable (<1 ng/ml) in 40.5%, low (1.1–2.5 ng/ml) in 5.4% and high (54.3–155000 ng/ml) in 54.1% of samples. About 37.8% of FNAB results were benign, 35.1% non-diagnostic and 27% malignant.
All patients with undetectable FNATg had benign or non-diagnostic FNAB. On the group of malignant FNAB ($n=9$), eight had high levels of FNATg and one had a low level.
After excluding the non-diagnostic FNAB, we obtained 88% of concordant results of FNATg and FNAB.
None of the patients with positive thyroglobulin antibodies and malignant FNAB had undetectable levels of FNATg.
Conclusion
In a patient with discordant FNATg and FNAB (high FNATg; benign FNAB), a surgical intervention confirmed the presence of metastasis. In this context, FNATg was of inestimable value, as it prevented further investigation that would delay cervical lymphadenectomy.
Positive thyroglobulin antibodies did not appear to influence FNATg levels.
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P1102

The diagnostic accuracy of ultrasound-guided fine needle aspiration biopsy (FNAB) in thyroid nodules 3 cm and above in size and sonographic feature differences between malignant and benign nodules
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Introduction
Nodule diameter was considered to be related with thyroid cancer. Whether under ultrasound (US) guidance or not, FNAB has some limitations particularly in big nodules. In this study, we aimed to evaluate diagnostic value of US-FNAB in nodules bigger than 3 cm or more. We also compared ultrasonographical features of benign and malignant nodules.
Material and methods
Data of 267 patients operated for nodulary goiter were screened retrospectively. The study group consisted nodules with a diameter of 3 cm or more. Nodules with a diameter of less than 3 cm were considered as control group. Cytological results were classified as malignant, suspicious for malignancy, undetermined, benign and nondiagnostic.
Results
There were 144 (53.9%) patients in study group and 123 (46.1%) patients in control group. Malignancy was observed in 16% of study group and 42.3% of control group. In nodules smaller than 3 cm, US-FNAB had a sensitivity of 85.4%, specificity of 40.3%, positive predictive value (PPV) of 52.6% and negative predictive value (NPV) of 78.1%. In nodules bigger and equal to 3 cm, sensitivity of US-FNAB was 72.7%, specificity was 81.6%, PPV was 45.7%, and NPV was 93.3%. Among parameters that may be used to predict malignancy, nodule texture, margin regularity, echogenicity and presence of halo were significantly different between malign and benign nodules.
Conclusion
This study showed that increased nodule diameter is not related with limitations in diagnostic value of US-FNAB. We also found that malignancy rate was smaller in big nodules. This finding reflects the importance of true and rational diagnostic and clinical management while detecting malignancy and deciding surgery.
Keywords
Thyroid nodule, fine needle aspiration biopsy.
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P1103

Evaluation of whole-body scan, stimulated thyroglobulin after thyroxine withdrawal vs recombinant TSH administration according to the risk groups of tumor recurrence

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Background
Recombinant human thyroid-stimulating hormone (rhTSH)-stimulated serum thyroglobulin (Tg) (s-Tg) and (131)I whole-body scanning (WBS) are supposed to provide equal diagnostic information with thyroxine withdrawal (THW) at follow-up thyroid cancer patients without the symptoms of hypothyroidism. We aimed to compare the WBS and s-Tg levels after thyrogen injection and thyroid hormone withdrawal for tumor recurrence/persistance according to the risk groups.
Methods
The study included 150 patients (119 females, 31 males) prepared to diagnostic WBS with rhTSH or THW. Age, sex, WBS, sTg levels, recurrence and risk groups for tumor recurrence (ATA guidelines 2009) were assesed retrospectively.
Results
WBS was performed with THW in 86 patients and with rhTSH administration in 64 patients. Mean age and sex were not different between the groups.
According to the risk groups for tumor recurrence, 71 patients were in low-risk group. In this group, 39 patients were prepared with THW and 32 patients were prepared with rhTSH for WBS. Mean diagnosis age and RAI doses were not different between the THW and rhTSH groups.
The peak TSH, s-Tg and Anti-Tg were significantly higher after THW compared with rhTSH administration. Both methods were equally effective for detecting metastatic or residual disease in low-risk group.
In moderate–high risk group (n: 79), 47 patients were prepared with THW and 32 patients were prepared with rhTSH for WBS. Mean diagnose age and RAI doses were not different between the groups. The peak TSH, s-Tg and Anti-Tg were also significantly higher after THW compared with rhTSH administration. Both methods were equally effective for detecting metastatic or residual disease in moderate–high risk group.
Conclusion
Both methods were equally effective for detecting metastatic or residual disease.

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P1104

Stimulated serum Tg $\geq$0.285 ng/ml in anti-Tg(−) cases had 3.087 times increased likelihood of recurrence of differentiated thyroid cancer: a single center experience

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Thyroid cancers constitute 2% of all cancer cases most of which are differentiated. Tumor size, lymph node metastases and thyroglobulin levels (Tg) in the follow-up period are among the major factors responsible for recurrence. In this study, we aimed to review our series of differentiated thyroid cancers and establish the risks of recurrence.
This study was carried out through a retrospective analysis of 393 differentiated thyroid cancer cases that were diagnosed in our department between January 2000 and December 2010. The demographic characteristics of the study group are seen in Table 1.
In the patients who had initial pathological lymphadenopathy, the recurrence risk was 2.76 times compared to the cases without pathological lymphadenopathy ($P<0.001$). The recurrence rate of male cases is 21 (30.4%). The cases with capsule invasion had 2.01 times increased risk of recurrence ($P=0.002$). The average tumor diameter in the 82 cases which relapsed was 2.04 ± 1.63 cm, and the average tumor diameter in the 311 cases under remission was 1.45 ± 1.21. A 1 cm increase in the tumor diameter increased the recurrence risk by 25% ($P<0.001$). Two hundred and eighty of the cases (71.2%) were multinodular, 108 of them 38.6% were multifocal, and multinodularity increased risk of recurrence 1.93 times ($P=0.021$). A postoperative Tg (pre-radioactive iodine (RAI) ablation therapy) value $\geq$2 ng/ml increased the recurrence risk and mortality significantly ($P=0.003$, $P=0.04$ respectively). Stimulated Tg values $\geq$2 ng/ml 6–12 months following RAI treatment increased the risk of recurrence ($P<0.001$). Disease-free survival was also significantly shorter in this group ($P<0.001$). A suppressed Tg value $\geq$0.3 ng/ml in the 6–12 months period following the first whole-body scan was associated with relapse ($P<0.001$). Serum Tg $\geq$ 5.6 ng/ml in early postoperative period increased the risk of recurrence 2.38 times ($P=0.002$). Excluding postoperative anti-Tg (+) cases, stimulated Tg values >0.285 ng/ml had 3.087 times increased likelihood of recurrence ($P<0.001$).

Table 1 Demographic characteristics of the study group

	Number and percentage of patients
Recurrence group	82 (20.9%)
Remission group	311 (79.1 %)
Histopathological analysis indicated papillary carcinoma	362 (92.1%)
Follicular carcinoma	31 (7.9%)
AJCC-2002 staging system	
Stage I	328 (83.5%)
Stage II	32 (8.1%)
Stage III	27 (6.9%)
Stage IVA	6 (1.5%)
Female	324 (82.4%)
Male	69 (17.6%)

In our study, we established that tumor diameter, male sex, lymph node metastasis, stage III and higher stage, presence of capsule invasion, increase in the nodule size that was initially measured with ultrasonography, postoperative Tg values higher than $\geq$2 ng/ml, suppressed Tg higher than $\geq$0.3 ng/ml, stimulated Tg $\geq$2 ng/ml, stimulated serum Tg $\geq$0.285 ng/ml in anti-Tg(−) cases, serum Tg $\geq$5.6 ng/ml in the postoperative scan increased the recurrence risk. We recommend that these factors are kept under consideration in patient follow-up.

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P1105

Papillary thyroid carcinoma with focal hobnail features

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Background
Papillary thyroid carcinoma (PTC), the most common endocrine malignancy, is a tumor of indolent biological and clinical behaviour that usually has a good prognosis. Only a small percentage of patients are affected by aggressive variants of PTC: tall, columnar cell and diffuse sclerosing variants. These aggressive tumor subtypes are characterized by higher rate of local recurrence, regional and distant metastases than classic papillary PTC. Recently another clinically aggressive variant of PTC with hobnail features has been described. However prognostic significance of such focal hobnail component in classic papillary PTC has not been defined.
Cases report
In this study, we reviewed clinical, cytological, histological, immunohistochemical and molecular biological features of five classic papillary PTCs with focal 'hobnail' component. Tumors were diagnosed in two woman and three men from 14 to 49 years old (mean age was 31 years). Cytologically these cases were characterized by increased nuclear/cytoplasmic ration of tumor cells, appearance of 'a shoe nail', separated arrangement of cells in smears, hyperchromic nucleus, cystic changes (dystrophy and cells degeneration, formation of 'spherical' complexes of tumor cells). Prevalence of focal 'hobnail' component in histological sections of PTCs was less than 20% of all tumor cells. Tumor size variation ranged from 0.2 to 1.5 cm (mean size was 0.85 cm). Regional metastases of PTC into the neck lymphatic nodes (LN) were diagnosed in all five patients. The $BRAF^{T1799A}$ point mutation was detected in one of three cases analysed by direct sequencing of DNA extracted from frozen tumor tissue. The size of primary tumor with $BRAF^{T1799A}$ was 1.5 cm. In four of five patients, the cytological material was obtained preoperatively by FNA from primary thyroid tumor, and metastatic tumors in LN.
Conclusion
PTC with focal hobnail component is characterized by the high frequency of regional metastases at the moment of primary tumor manifestation. Obviously that prevalence of $BRAF^{T1799A}$ point mutation in PTC with focal hobnail features is similar as in classic papillary PTC without hobnail component.

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P1106

Serum calcitonin, thyrotropin, and goiter

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Objective
Recent papers reported that basal calcitonin (CT) level may be related to thyroid volume. This study aims to evaluate if this finding is confirmed in patients undergoing ultrasonography-guided fine-needle aspiration cytology (FNAC) for thyroid nodules.
Design
Retrospective university-center study.
Methods
From February 2010 to September 2012, 561 patients underwent ultrasonography-guided FNAC and a complete evaluation including basal serum FT4, FT3, TSH, CT and estimation of thyroid volume.
Results
The mean thyroid volume was 21.10 ± 9.58 ml in males and 13.42 ± 6.48 ml in females ($P < 0.001$). Thyroid was found to be atrophic in 18 cases and goiter was diagnosed in 128 patients. A linear regression analysis was performed between serum CT levels and thyroid volume, showing a weak direct relationship ($R2 = 0.023$, $P < 0.001$). There is no correlation between serum TSH and CT levels. In patients grouped according to morphologic diagnosis (atrophy, normal volume and goiter), CT levels are slightly higher in the high-volume groups: the mean value was 2.02 ± 0.09 in the atrophy group, 2.86 ± 1.73 in the normal volume group, and 3.00 ± 1.66 in the goiter group ($P = 0.02$). When males and females are computed separately, the statistical significance is lost.
Conclusions
The small difference in basal CT levels is probably due to a genetically determined higher thyroid volume and increased number of C-cells rather than to acquired goiter. Gender may act as a 'surrogate marker' of thyroid volume and the application of a gender-specific cut-off can probably overcome this issue.

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P1107

Incidental thyroid cancer frequency in total thyroidectomy for the graves disease patients and the effect of the presence of nodules on malignity rates

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Introduction
Our objective for the current review was to inquire the thyroid cancer frequency rate within the patients who have had total thyroidectomy procedure for definitive Graves' disease treatment and to evaluate the contribution of the presence of nodules to malignity rates.
Materials and methods
The present study was conducted by scanning the data of 214 patients who were applied total thyroidectomy for permanent Graves disease treatment, retrospectively. The participants were assorted into sub groups as benign nodule group, malignant nodule group and no nodule group and afterwards evaluated. The groups were also compared in regards to demographic characteristics and laboratory data.
Findings
Fourteen of the 214 cases were diagnosed with cancer. The malignity prevalence was determined as 6.5%. The sex distributions were found to be statistically similar between the malignant and benign nodule groups ($P = 0.776$). The mean age of the malignant nodule group was significantly higher than the benign nodule group ($P = 0.042$). No significant difference seemed to be between the benign and the malignant nodule group in regards to median TSH receptor antibody levels ($P = 0.134$). The cases were divided into two in regards to nodule presence. Pre-operational USG revealed nodules in 19 cases. Five of these patients had malignity. The malignity frequency was significantly higher in the nodule positive group than the no nodule group (26.3 vs 4.6% relatively, $P = 0.004$). There was also no statistically significant TSH receptor antibody, Anti-TPO antibody titres and thyroid functioning difference between these groups ($P > 0.05$)
Conclusions
We have determined the incidental thyroid cancer frequency in Graves' disease patients population as 6.5%. Malignity rate grew higher when co-morbid nodules were also present (26.3%). We recommend that when total thyroidectomy patients are the case who is treated surgically for the Graves' disease, especially if nodules are present, malignity risk must be taken into consideration.

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P1108

Detecting somatic oncogene mutations in FNA samples of cold nodules in Hungary

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Cold nodules are one of the most common findings on scintigraphic examinations of the thyroid gland. About 5–10% of these nodules turn out to be histologically malignant. Our aim was to examine some somatogenetic alterations associated with thyroid cancer in FNA samples of the thyroid. These alterations included single nucleotide mutations (BRAF, HRAS, NRAS, KRAS) and genetic translocations (RET/PTC1, RET/PTC3, PAX8ex7/PPARgamma, PAX8ex9/PPARgamma). The SNPs were tested by real-time PCR with fluorescence melting curve analysis and the rearrangements were detected by Taqman probe-based quantitative real-time PCR. We have analyzed 250 consecutive FNA samples. In the examined samples, we found different genetic alterations (four BRAF, one NRAS, seven HRAS mutations and one RET/PTC3 rearrangement) in 13 of these cases. By cytology and histology, 19 cases were classified as malignant, from which we identified genetic alterations only in 5 (26.32%). In eight cases out of the 13 genetic alterations, no sign of cytological malignancy could be seen at the time of the study. In the 11 samples from 19 with papillary cancer, four BRAF (36.36%) and one RET/PTC3T (9.1%) mutations were detected. The rest of malignancies from 19 (follicular lesions and one medullary cancer) showed no genetic alterations. No PAX8/PPARgamma rearrangements were demonstrated in the 250 samples. These data are not in complete accordance with published information. This fact might be due to several factors including the differences in iodine supply in different geographical areas.

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P1109

Predictive value of preablation stimulated thyroglobulin in differentiated thyroid cancer

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Background
Previous studies have suggested that serum thyroglobulin (Tg) levels at the time of 131I remnant ablation just after thyroidectomy, could be a prognostic tumor marker in differentiated thyroid cancer (DTC).
Objective
The aim of this study was to evaluate if serum preablation Tg in the earliest postoperative period could be useful for predicting persistence or recurrence of disease.
Methods
This study was a retrospective analysis. We included 160 patients with DTC who underwent total thyroidectomy and received ablative therapy with I131 from January 2000 to December 2011. A stimulated Tg was measured just at the time of the first 131I therapy (preablation-Tg). We correlated this value with a stimulated Tg-control (control-Tg) measured at the time of the diagnostic whole-body scan, performed approximately 6–12 months after preablation-Tg. Exclusion criteria were positive Tg antibody ($n = 9$) and microcarcinoma ($n = 26$). Follow-up was

performed according to the American Thyroid Association Guidelines published in 2009. The minimum follow-up period was 14 months.
Results
Sixty-three of 77 patients (82%) with preablation-Tg $\leq$3 ng/ml showed undetectable ($\leq$1 ng/ml) control-Tg and 37 of 48 (77%) patients with preablation-Tg $>$3 ng/ml showed detectable ($>$1 ng/ml) control-Tg ($P<0.001$). At follow-up, 72 of 91 (79%) with preablation-Tg $\leq$3 ng/ml showed remission and 20 of 28 patients (71%) who showed persistence/recurrence, had a preablation-Tg $>$10 ng/ml ($P<0.001$). The negative predictive value for recurrence in patients having preablation-Tg $\leq$3 ng/ml was found to be 98.4% (73 of 77 patients, $P<0.001$).
Conclusion
Preablation-Tg levels correlated well with the control-Tg levels. A preablation-Tg $\leq$3 ng/ml is a favorable prognostic factor in DTC.

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P1110

Concurrent follicular and columnar cell variants of papillary thyroid carcinoma characterized by different BRAF status and metastatic properties: a case review

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Papillary thyroid carcinoma (PTC) is a malignant tumour of follicular cell origin that is characterized by a broad diversity of histological variants. A genesis of multifocal PTC may be explained by both intrathyroid tumour dissemination and multiple focuses of tumour origination. A combination of two different focuses of PTC characterized by various molecular alterations of tumour cells in one thyroid could be rarely occurred. Follicular variant has similar clinical behaviour and prognosis as classic papillary PTC. Columnar cell variant was initially defined as an aggressive lesion by Evans in 1986.
Here, we present a case review of concurrent follicular and columnar cell variants with molecular analysis of *BRAF* status in tumour cells of different PTC histotypes.
Female of 62 years old was admitted to the Medical Radiological Research Centre with complaints of pain in a right thigh. A tumour of right femoral bone was diagnosed by X-ray examination. A core tumour biopsy revealed metastasis of thyroid carcinoma that cells were immunopositive for thyroglobulin and TTF-1. Thyroid ultrasonography showed two nodular lesions in right lobe and isthmus. Histological study of the thyroid after thyroidectomy revealed follicular variant in isthmus and columnar cell variant of PTC in right lobe. Histological review of resected right femur with metastatic tumour showed columnar cell type of PTC. Molecular study of DNA extracted from two different tumours revealed wild type status of *BRAF* in follicular variant and $BRAF^{T1799A}$ point mutation in columnar cell variant.
Columnar cell variant of PTC is more aggressive type of thyroid carcinoma in a patient with two concurrent PTC variants (follicular and columnar cell). In contrast with follicular variant columnar cell variant of PTC was characterized by distant bone metastases and $BRAF^{T1799A}$ point mutation.

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P1111

Thyroglobulin as an early marker of persistent/recurrent disease in patients with differentiated thyroid carcinoma (DTC)

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Introduction
Several clinical and molecular markers can be used to establish risk at the time of diagnosis in DTC. Thyroglobulin (Tg), which is essential in follow up, could be an early marker to identify risk patients. Around 25% of patients require 18 months or longer to have indetectable Tg. The presence of Tg antibodies (TgAb) invalidate the follow up of DTC via Tg and this is usually associated with a poor prognosis.
Aim
To evaluate the role of Tg after surgery and before radioiodine treatment, and the presence of TgAb as markers of persistent/recurrent disease in DTC.
Patients and methods
Retrospective study of 165 patients with DTC (1997–2010): women 78,2%, age at diagnosis 47.9+/- 16,26, medium follow up 4.8+/-3.74 years.
We performed a multivariate (logistic regression) analysis including Tg after surgery and before radioiodine treatment and other known prognostic variables. We divided Tg into tertile (cut-off: 1.7 ug/l, 13.6 ug/l) excluding TgAb positive at first before adding it later. We performed a ROC curve to identify cut-off level of Tg. Statistical analysis was performed with SPSS 19.0.
Results
In the multivariate analysis excluding TgAb positive, Tg in higher tertil is the unique predictive variable of persistent/recurrent disease at 18–24 months (RR 21.67). Adding positive TgAb, the predictive variables are Tg (RR 21, 04) and presence of TgAb (RR 112). The ROC curve shows a cut-off of Tg after surgery and before radioiodine treatment of 5,55 ug/l that identifies persistent/recurrent disease (S 81, E 57,5, NPV 92,5 and PPV 31,5% (AUC 0.809)).
Conclusions
In this serie, Tg value after surgery and before radioiodine treatment and the presence of TgAb are predictive variables of recurrent/persistent disease at 18–24 months. Tg has a predictive value and could be a useful marker to stratify risk at the time of diagnosis.

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P1112

Ultrasound guided enolization as a therapeutic alternative in local recurrences of papillary thyroid carcinoma

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Introduction and objectives
Multiple surgeries are associated with many complications in the local recurrence of papillary thyroid carcinoma. Our objective is to evaluate the efficiency of the enolization in the recurrent metastatic cervical lymph nodes in patients with limited nodal disease.
Material and methods
We studied 13 patients (six males and seven females) with a mean age of 43 ± 14.4 years with papillary thyroid carcinoma intervened (total thyroidectomy) . In our follow-up, they present nodal recurrence (23 lymph nodes). Seven had previous lymphadenectomy (1–3) and they had received a mean I-131 dose of 238.46 ± 71.25 mCi. In four cases, the anti-Tg Ab were elevated where the PET was positive. All of them showed pathological levels of Tg-FNAB (1.7–15 342 ng/ml). Guided by ultrasound, we injected absolute ethanol (20–30% of total lymph node). Were required for 1–3 enolizations spaced in 3–4 months until the absence of vascular flow.
Results
The mean nodal volume was 0.155 ± 0.071 cm^3. The initial mean thyroglobulin ON was 5.70 ± 5.08 ng/ml. We observed a nodal volume reduction with no vascular flow in all cases with an average volume of 0.075 ± 0.054 cm^3 postenolization (-0080 cm^3, $P<0.0001$). We got a reduction$>$ 30% in 18 lymph nodes, $>$50% in 13, $>$70% in nine and disappearance in six. We also observed a decrease in average thyroglobulin levels to 3.47 ± 4.17 ng/ml (-2.23 ± 1.90, $P<0.01$). In six of nine patients, the reduction in the levels of thyroglobulin was $>$50%. All without evaluate the cases with Tg Ab positive. No significant adverse reactions were observed.
Conclusions
The ultrasound-guided enolization is presented as an excellent alternative to surgery for local recurrence of thyroid papillary carcinoma in selected cases where surgery is not recommended.

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P1113

Liquid preparation cytology for evaluation of thyroid nodules

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Introduction
FNA (fine needle aspiration) is a highly sensitive and specific procedure in evaluating of thyroid nodules. There are several options for processing the FNA specimen such as direct smears, cell block preparations (CBP) and liquid base preparations (LBP); the latter has become more popular due to its easy transport, fast protocol and also the possibility to study different parts of the specimen. In this study, we compare the results of cell block and liquid base preparation in the same samples.
Design and methods
FNA performed for 80 patients with minimally 1 cm diameter solitary nodule or a prominent nodule on a multinodular background (excluding hot nodules), the obtained samples used to prepare direct smears, cell blocks and liquid preparations. The microscopic results recorded considering the following: cellularity, blood, colloid and macrophages.
Results
Forty-five of the samples (56.25%) show the same results in both CBP & LBP which confirmed direct smear findings. In 11 samples (13.75%), the CBPs, and in 24 samples (30%) the LBPs were more informative and diagnostic.
Conclusion
Totally in about 70% of cases, LBC method had sufficient diagnostic microscopic data so the liquid base method should be trusted due to its easier procedure & cleaner slide background. It could be used instead of CBP and in association with direct smears for precise evaluation of thyroid nodules.

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P1114

Prevalence of hypovitaminosis D in patients with papillary thyroid carcinoma (PTC)

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Introduction
The correct 25-hydroxy vitamin D (25(OH)D) level plays a key role in the proper supply of vitamin D for the organism. Vitamin D deficiency may lead to an increased risk of development of various neoplasms, including papillary thyroid carcinoma (PTC).
Aim
Evaluation of the prevalence of vitamin D deficiency in patients with PTC and analysis of the relation between 25(OH)D levels and age, sex, place of living, severity of the disease, and presence of the CHEK2 mutation
Material
The study consisted of 168 patients with PTC; F:149 (88.7%), M: 19 (11.3%), aged 19–78 years. Mean age 52.3 (SD ± 13.6) years.
Methods
Exams were conducted during autumn. Plasma levels of 25(OH)D were measured by the RIA method. Prevalence of vitamin D deficiency in the studied group and the relation of the 25(OH)D levels, prevalence of 25(OH)D deficiency and age, sex, place of living, severity of the disease, and presence of the CHEK2 mutation.
Results
25(OH)D < 30 ng/ml deficiency was present in 101 patients and extremely low vitamin D < 10 ng/ml in three patients.
Vitamin D deficiency was present in 62.1% of inhabitants of cities and 65.1% of inhabitants of villages, 63.3% women and 63.2% men, 63.8% patients in I stadium of the disease, 58.8% – II, 57.7% – III, 85.7% – IV, and 56.2% patients with CHEK2 mutation and 69.5% without mutation.
No correlations were found between 25(OH)D levels, prevalence of 25(OH)D deficiency and age, sex, place of living, severity of the disease, and presence of the CHEK2 mutation.
Conclusions
More frequent 25(OH)D deficiency due to CHEK2 gene mutation or severity of the disease was not found. Results suggest the need for prophylaxis programme of the vitamin D deficiency in patients with PTC, but do not allow to point to vitamin D deficiency as the factor potentially influencing the onset and course of the disease.

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P1115

Primary thyroid lymphoma – report of four cases

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Introduction
Primary thyroid lymphoma (PTL) is responsible for 0.6–5% of all cases of thyroid cancer and less than 2% of all extranodal lymphomas. PTL is more common in elderly women and are commonly of B-cell origin. Current treatment regimens for PLC consist of chemotherapy and external beam radiation. Surgery plays an important role, including the control of symptoms in large obstructive lymphomas.
Aims and methods
Retrospective data review of pts with PTL diagnosis followed at our Institution's Endocrinology Department.
Case reports
Four female patients (pts), with 53–78 years (median 65). Three pts presented with a multinodular goiter, one pt with a solitary thyroid nodule and all had compressive symptoms. Thyroid function tests were normal at presentation and lymphocytic thyroiditis (LT) had been previously diagnosed in one pt. Cytological exam was suspicious for lymphoma in one case. Two pts were submitted to total and two pts to subtotal thyroidectomy. Histopathology revealed diffuse large B-cell lymphoma in two pts, a marginal zone B cell lymphoma and a Burkitt's lymphoma. LT was described in two patients' report. Three patients received combination chemotherapy (CHOP regime in two pts and BFM protocol in one pt). One pt received local radiotherapy. Imaging modalities revealed other areas of involvement in two pts. Three patients showed complete response to treatment and in one pt chemotherapy had to be stopped because of its toxicity.
Discussion
Primary lymphoma is a rare type of thyroid cancer and its cytological diagnosis can be complex because of the discrimination from LT, which is associated with an increased risk of TPL. Thyroid lymphoma should be suspected in the presence of a rapidly enlarging neck mass causing compressive symptoms, mostly in elderly woman with a LT diagnosis. The disease can often be cured without the need for extensive surgery if recognized early.

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P1116

Treatment, follow up and prognostic factors of papillary microcarcinoma

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Introduction
The incidence rate of papillary thyroid microcarsinoma (PTMC) has almost doubled during the recent years but treatment and follow up is still a matter of debate. In this study we aimed to analyze clinical and histopathological risk factors at the time of diagnosis and to observe their implications for treatment, follow up and prognosis.
Patients and methods
Two hundred forty-eight PTMC patients were included in this study. The age, sex, the method of diagnosis (incidental or with a clinical suspicion), cervical lymph node metastases and relapse and/or distant metastases during follow up were retrospectively recorded.

Results
Two hundred one were female and 47 were male. Total thyroidectomy was performed in all patients. All of the patients had postsurgical radio-iodine ablation treatment. When compared according to tumor size ($\leq$5 mm vs. $>$5 mm), bilateral involvement, vascular invasion, capsular invasion, extrathyroidal extension and lymph node metastases were significantly more frequent in the patients with tumor size $>$ 5 mm (P values $P<0.046$, $P<0.021$, $P<0.001$, $P<0.003$, $P<0.000$ respectively). Diagnosis after a clinical suspicion and thyroglobulin (TG) value were found to be associated with lymph node metastases at the end of the multiple logistic regression analysis. The relevant TG value was 7.98 ng/ml with a sensitivity of 57.14% and specificity of 83.17%. Relapse was associated with TG value and lymph node metastases at the time of diagnosis.
Conclusion
Local relapse is significantly associated with lymph node metastases at the time of diagnosis. Regarding the treatment of PTMC our approach is to perform total/near total thyroidectomy and than RAI treatment. We think that the low relapse in this study is related with our therapeutic approach. On the other hand, PTMC should not be regarded as a relatively benign disease when our patients with lymph node metastases, local relapse and distant metastases were taken into consideration.

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P1117

Macrofollicular variant of papillary thyroid carcinoma: an uncommon entity

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Background
Macrofollicular variant (MV) is a rarely seen variant of papillary thyroid carcinoma (PTC). We herein present clinical characteristics of four patients carrying this variant of PTC. Case 1: 62-year-old female patient underwent total thyroidectomy (TT) due to euthyroid multinodular goiter (MNG) with a cytology of 'indeterminate nodule'. The pathology revealed 'papillary thyroid micro-carcinoma MV with a diameter of 8$\times$6 mm, without capsule or lymphovascular invasion (LVI)'. Case 2: 61-year-old female patient underwent TT due to toxic MNG. Pathology revealed 'thyroid papillary microcarcinoma MV with a diameter of 9$\times$9 mm, without capsule or LVI'. Case 3: 60-year-old man underwent TT due to euthyroid MNG with pressure symptoms on his neck. Pathology revealed 'bilateral multisentric PTC; a focus of MV with a diameter of 2$\times$2 cm in the right lobe; two foci of classical variant with diameters of 3$\times$2 and 2$\times$2 mm in the left lobe'. No local/distant metastasis were identified in cases 1, 2 and 3. Case 1 and 2 were started TSH suppression therapy and case 3 received radioactive iodine (RAI) and suppression therapy afterwards. Case 4: 50-year-old male patient with a previous diagnosis of metastatic PTC after left cervical lymph node (LN) excision, underwent TT, central and lateral neck dissection. Pathology revealed 'PTC MV focus of 2.7 cm with capsule and LVI, and metastatic LN on left lateral neck (biggest in diameter 5 cm) and left paratracheal compartment (5.5 cm in diameter)'. No distant metastases were identified. He has been referred for RAI ablation therapy.
Conclusion
Macrofollicles in PTC-MV may cause diagnostic pitfalls during cytology which may be incorrectly defined as benign goiter. The presence of macrofollicles $>$ 200 μm in $>$50% of the cross-sectional area is pathognomonic for PTC-MV. Despite its rarity and benign course, these patients should be evaluated for local/distant metastases.

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P1118

Postoperative stimulated thyroglobulin levels in thyroidectomized patients with differentiated thyroid cancer

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Introduction
The presence of undetectable serum levels of stimulated thyroglobulin (Tg) with negative imaging tests is the criterion used to define remission in low-risk differentiated thyroid cancer (DTC). Most guidelines recommend 131-I ablation after thyroidectomy to remove any possible residual thyroid tissue. Our aim was to know how often undetectable Tg levels are achieved before ablation to avoid unnecessary radiation treatments.
Patients and methods
One hundred and eighteen patients (106 women and twelve men, age 15–83 years) with low-risk DTC who underwent total thyroidectomy from 2005 to 2011were included. Tg, anti-Tg antibodies (TgAb) and TSH were analysed before and the fifth day after a dose of recombinant human TSH administration. They were measured in an Inmmulite® 2000 (Tg functional sensitivity: 0.9 ng/ml) 6–18 weeks after the surgery. Subsequently, a dose of 100 mCi of 131-I was administered to all patients and other possible treatments were applied when necessary. A correlation between postoperative stimulated Tg concentration and clinical situation at the end of the follow-up (25.6$\pm$15.6 months) was performed.
Results
Eleven patients with positive TgAb were excluded. Tg levels were undetectable in 50 patients (47%), 1–10 ng/ml in 42 (39%) and $>$10 ng /ml in 15 (14%). At the end of the study, there was no evidence of recurrence in the 50 patients with undetectable postoperative Tg. Remission criteria were met in 39/42 and 9/15 patients with Tg between 1–10 and $>$10 ng/ml, respectively.
Conclusions
In near half of the patients with low risk DTC 131-I therapy is not necessary to achieve undetectable Tg levels after total thyroidectomy. An undetectable postoperative stimulated Tg predicts the absence of recurrence during the long term follow-up. 131-I ablation should not be necessarily performed in all the patients with low-risk DTC.

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P1119

Angiosarcoma of the thyroid gland: a case report

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Angiosarcoma of the thyroid is a rare and aggressive primary malignant tumor of the thyroid: it rarely occurs in that organ and most of these occur mainly in patients from the mountainous Alpine regions. We report a case of 68-year-old female who presented at our hospital with a rapidly enlarging neck mass associated with compressive symptoms. On clinical examination, the thyroid gland appeared firm during the acts of deglutition. The results of thyroid function tests were for subclinical hyperthyroidism. Ultrasound examination and CT scan showed multinodular goitre with a suspicious nodule from the right thyroid lobe. Fine needle aspiration cytology of the thyroid was suggestive of anaplastic thyroid carcinoma. A total thyroidectomy was performed. Grossly, the right lobe and left lobe measured 8$\times$7$\times$4 cm and 8$\times$8$\times$7 cm. Macroscopically, the cut surface showed a bulging solid hemorrhagic dark red mass, measuring 4.8$\times$3.2$\times$2.5 cm, at the lower pole of the right lobe. The immunohistochemistry showed CD31 and CD34 positivity and thyroglobulin, calcitonin, and TTF-1 negativity indicating an angiosarcoma. The patients died within 2 months following up from pulmonary hemorrhage.
In conclusion, this case illustrates that thyroid angiosarcoma is a distinct entity and should be considered and included as a differential diagnosis of poorly differentiated thyroid neoplasms also outside the mountainous Alpine regions.

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P1120

MIBI – spect scintigraphy in the presurgical assessment of thyroid lesions with indeterminate cytology

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Introduction
Thyroid lesions with indeterminate cytology represent about 15% of all thyroid biopsies with the risk of malignancy 15–30%. So the identification of low-risk patients in this group is very important.
Few studies evaluated thyroid Tc-99m MIBI [MIBI] scan as a tool for work-up of indeterminate cytology thyroid lesions. The negative predictive value of this test in excluding malignancy appears to be high (95%). But it is not used routinely. This study evaluated the role of MIBI scan in the assessment of these lesions.
Methods
Patients with indeterminate cytology and Tc-99m Pertechnetate thyroid scan-cold nodules were included in the study during the period of January 2009 to August 2012 retrospectively. MIBI scan was offered to the patients who initially did not accept surgery as a treatment option.
Results
Sixteen patients were included in the analysis (three men and 13 women, age: 52 ± 15 years, lesion size 21 ± 8 mm). All patients had suspicion for follicular lesion cytology (Bethesda 3). Three patients (18.7%) had 'cold' lesions on MIBI scan and surveillance was offered. The follow-up of 12–36 months showed no sonographic and clinical changes in these patients. Thirteen patients had 'hot' lesions. Nine patients (69%) were operated and four (44%) of them had malignancy (two papillary carcinoma, one follicular variant of papillary carcinoma and one follicular carcinoma). Four patients with 'hot' MIBI nodule refused operation and continued the follow-up during 6–12 months with stable sonographic characteristics.
Conclusions
In our small sample size experience, we were able to define a low risk of malignancy in 18.7% of the patients with MIBI scan assessment. This protocol helped to decrease unnecessary surgical intervention from 67 to 56%. Larger studies with longer follow-up are needed to validate the amount of 'cold' lesions in this unique subgroup and mainly the long-term consequences of the patients with 'active surveillance' policy.

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P1121

Clinical and biochemical criteria for the prognosis of small medullary thyroid carcinomas

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Introduction
Recently, small medullary thyroid carcinomas (sMTCs) (≤ 1.5 cm) are frequently diagnosed, occasionally as incidental findings in surgical and autopsy specimens. Their clinical course varies. We examined whether tumor size is a predictor of clinical behavior.
Patients and methods
One hundred and one sMTC patients (36.6% males, 47.1% familial) followed-up for 0.9–35 years. Patients were classified according to tumor size (cm) in group1: 0.1–0.5 (n = 25), group2: 0.6–0.8 (n = 22), group3: 0.8–1.0 (n = 23), group4: 1.1–1.5 (n = 31).
Results
Familial cases did not differ from sporadic ones concerning stage at diagnosis or outcome. Preoperative and postoperative calcitonin levels were positively associated with tumour size ($P < 0.001$). At diagnosis, capsular and lymph node invasion were more frequent in groups 3 and 4 ($P < 0.022$, $P < 0.001$ respectively). The stage at diagnosis was more advanced and the outcome less favourable with increasing tumour size ($P < 0.004$). Group 1 and 2 patients were more frequently cured (group 1: 88%, group 2: 86.7%, group 3: 72.7%, group 4: 51.7%, $P = 0.009$). The 10-year probability of lack of progression of disease according to tumour size did not differ significantly between the four groups (group 1: 96%, group 2: 100%, group 3: 100%, group 4: 81.5%, $x^2 = 4.61$, $P = 0.2$, log rank); it differed marginally between patients with tumour 0.1–1.0 and 1.1–1.5 cm (98.5 and 81.5%, $x^2 = 4.15$, $P = 0.042$, log rank). In the subgroup of microMTCs (≤ 1.0 cm) patients with microMTC ≤ 0.8 had less advanced stage at diagnosis compared to 0.9–1.0 cm (stage I and II: 89.4 vs 66.7%, stage III: 8.5 vs 33.3% and stage IV: 2.1 vs 0%, $P = 0.032$). No differences in the outcome were found between microMTC subgroups.
Conclusion
The probability of 10-year disease progression slightly increases in > 1.0 cm sMTCs. In microMTCs although the stage is less advanced in microMTCs ≤ 0.8 cm, the outcome is similar to larger microMTs (0.9–1.0 cm). Thus tumor size may be of clinical importance only in patients with MTCs > 1 cm.

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P1122

Diagnostic validity of thyroid ultrasonography in thyroid nodules

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Introduction
A thyroid ultrasound scan is recommended in the assessment of any suspected thyroid nodule. The ultrasound characteristics of nodules have been shown to be useful in assessing their malignant potential. The aim of this study is to determine diagnostic validity of thyroid ultrasound in differentiating benign and malignant thyroid nodule.
Material and Methods
It was cross-sectional study, conducted at XXXX Hospital from August 2011 to July 2012. All patients of either gender with thyroid nodules referred for ultrasound thyroid and FNAC were included. Patients with known thyroid malignancy, pure cystic lesion, indeterminate, non-diagnostic, suspicious finding in cytology without subsequent surgery were excluded. Ultrasonography was performed by radiologists with Toshiba US machines. The ultrasound parameters were assessed and compared with FNAC results in all nodules. Diagnostic validity of each ultrasound feature was calculated. Study was approved from Ethical Review Committee of Hospital.
Results
Total 101 patients were included in the study on this basis of availability of ultrasound images, pathology report and after exclusion. Mean age of patients was 43 ± 13 years (range 15–73 years) and $n = 81$ (80%) were females. Among 101, $n = 96$ benign and $n = 5$ nodules were malignant on histocytopathology. The sensitivity and specificity of each ultrasound feature in predicting malignancy were microcalcification, 80 and 68%; hypoechogenicity, 80 and 52%; ill-defined lobulated margin, 40 and 96%; solid, 80 and 40%; taller than wider, 50 and 63% respectively. Each US feature have negative predictive value ranges from 95 to 98%.
Conclusion
Identification of microcalcification, hypoechogenicity and solid with ill-defined margins thyroid nodules on ultrasound is helpful in diagnosing thyroid malignancy and warrants urgent diagnostic biopsy.

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P1123

Histopathologic characterization of differentiated thyroid carcinoma in an area of Basque country

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Background
The most common type of thyroid cancer is the well-differentiated thyroid carcinoma (DTC) and that includes papillary (PTC) and follicular carcinoma (FTC). The PTC would represent 50–90% and the FTC over 15–30%. Within the PTC, the most frequent is the classical variant (over 80%), followed by follicular variant (10%) and the rest of variants ($< 10\%$). Furthermore, there are differences between PTC and FTC in age at diagnosis, local extension, multicentricity and the presence or absence of lymphadenopathy.
Our aim was to analyze the prevalence of the different variants of CDT in an area of the Basque Country and the histopathologic characteristics of the sample.
Methodology
We studied the medical records of 110 patients diagnosed with differentiated thyroid cancer, obtained from hospital tumor registry between the years 2005–2012. Using SPSS we performed a descriptive analysis of the sample and compared means.
Results

$N = 110$	PTC = 89 (81%)	FTC = 21 (19%)	P
Age at diagnosis	54 ± 17	51 ± 19	n.s.
Average size	15.7 ± 13	33.8 ± 16	< 0.05
Multicentricity	31%	15%	n.s.
Dedifferentiation	2%	10%	n.s.
Local extension	18%	10%	n.s.
Lymphadenopathy	20%	0%	< 0.05
Histotypes	Classical variant 38% Follicular variant 38% Diffuse sclerosing 5% High cell 1% Columnar cell 1% Clear cell 0% Mixed 17%	Minimally invasive 67% Widely invasive 14% Oncocytic 14% Trabecular 5%	

No significant differences among the different histotypes of each group in terms of age at diagnosis and tumor size were found. There was also no correlation between size and age at diagnosis.

Conclusions
Unlike described by the literature, the follicular variant PTC is more frequent in our area. Tumor size at diagnosis in the FTC is significantly higher than in the PTC. The PTC was presented with lymphadenopathy at diagnosis in a rate of 20%, unlike the FTC.
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P1124

A rare case of nonsecretory medullary thyroid carcinoma
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Introduction
Medullary thyroid carcinoma (MTC) is a rare form of thyroid cancer, making up about 3–5% of all cases. It is characterized by the synthesis and secretion of calcitonin (Ct). Measurement of Ct has low specificity but is a highly sensitive method for the detection of MTC. MTC with normal or only slightly elevated Ct is a rare occurrence and there are few such reports in the literature.
Case report
A 61-year-old male patient was referred to the Endocrine consultation for evaluation of a solid, solitary, right lobe thyroid nodule, 38×30 mm in diameter. There were no suspiciously enlarged nodules on the neck sonogram. The nodule had fisrt been detected 2 years before and grew slowly since then. Fine needle aspiration (FNA) revealed a benign cytopathology. Serum TSH and FT4 were normal, with increased anti-peroxidase antibodies (826 IU/ml). Serum calcitonin was 15.4 ng/l and CEA was 2.4 µg/l. The patient underwent right lobectomy. Pathology analysis revealed MTC. There was no vascular nor capsular invasion. Immunohistochemistry was positive for synaptophysin and chromogranin A, and in very few cells for CEA and Ct. The frozen section specimens were sent to another Pathology Department of a public Oncology Hospital, which confirmed the diagnosis of MTC. Postoperative Ct level was <0.2 ng/l. Habitus marphanoid and mucosal neuromas were not apparent. Urinary cathecholamines and metanephrines were normal, as were serum calcium and PTH. There was no family history of endocrine disease. The results of the genetic testing of the *RET* gene are still pending.
Conclusions
The features of the sonogram and FNA test and the minimally increased level of Ct in the context of goiter and auto-immune thyroiditis did not raise suspicion of thyroid cancer. This is one of the rare cases described in the literature of MTC with almost normal Ct.
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P1125

Coexistent medullary thyroid carcinoma and multifocal papillary thyroid microcarcinoma in a patient with chronic autoimmune thyroiditis
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Introduction
The association between medullary (MTC) and differentiated thyroid cancer (DTC, with the most frequent form of papillary thyroid carcinoma, PTC) is rare and can be observed in two main settings: a collision tumor (that is, a tumor with two separate and different components) or a mixed tumor showing dual differentiation.
Case report
A 58-year-old woman, affected by euthyroid multinodular goiter came to our observation for a second opinion regarding the surgical indications for their goiter. The thyroid antiperoxidase antibodies titer was elevated, ATPO: 40.7 UI/ml (normal values <5.61). An ultrasound scan revealed diffuse thyroid enlargement with a suspicious nodule (hypoechoic, with irregular margins, calcifications and intranodular hypervascularization) of 9.8/11.7 mm, recently described in the upper third of the left thyroid lobe. In both lobes, other micronodules hypo- or isoechoic were also described. The serum calcitonine (CT) was 78, repeated 82 pg/ml (normal values $\leq$ 2 pg/ml) and carcinoembrional antigen (CEA) was also elevated. The fine-needle aspiration cytology was nondiagnostic and patient was referred for surgery for total thyroidectomy with central compartment neck dissection. The histological examination confirmed the diagnosis of medullary thyroid cancer, showing a lymphocytic intratumoral infiltration and multiple occult follicular variant of papillary microcarcinoma in both lobes. One of this microcarcinoma was adjacent to the medullary carcinoma. No lymph node metastases were found. The serum levels of CT and CEA decreased to normal after the operation. Molecular analysis of the RET proto-oncogene was performed.
Conclusions
In our case, the simultaneous occurrence of MTC and multifocal PTC has the features of a collision tumor, although the close relationship of one of the foci of PTC with MTC has rise the hypothesis of a mixed tumor.
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P1126

BRAF V600E mutation in papillary thyroid cancer: clinical and pathological features. Is there any role in tailoring initial treatment?
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Introduction
BRAF (V600E) mutation is the most frequent detected genetic change in papillary thyroid cancer (PTC) and its presence has been related to aggressive clinical and pathological features. Lymph node metastases (LNMx) are common in PTC and are associated with an increase in loco-regional recurrence. However, prophylactic lymph node dissection is not routinely performed because of high rate of surgery complications. Therefore, there is a need to find a good marker to decide the extent of initial surgery.
Methods
We evaluated 31 patients (77.5% females) with pathological diagnosis of PTC. All of them underwent total thyroidectomy and 28 central lymph node dissection, being in 20 prophylactic. DNA was extracted from neoplastic cells and BRAF mutation was detected by PCR and sequencing. Analysis included age, preoperatively TSH, tumour size, multifocality, extrathyroidal extension (EET), LNMx, histological subtype, clinical stage and ultrasound features.
Results
The prevalence of the BRAF mutation (BRAF+) in our patients was 51.6%; 48.4% were negative (BRAF−). According to sex, 57% males and 45% females were BRAF+ ($P=0.68$). Mean age was 46.8 years in BRAF+ vs 55.4 in BRAF− ($P=0.28$); mean tumour size was 17.8 mm in BRAF+ vs 22.8 in BRAF− ($P=0.35$). Multifocality was present in 66.6% of BRAF− and 50% of BRAF+ ($P=0.47$). EET was found in 33% of BRAF− vs 56.2% of BRAF+ ($P=0.28$)). In 69% of BRAF+, classic variant of PTC was diagnosed, whereas 6% corresponded to follicular variant ($P=0.07$). Mean TSH level was 3.97 mU/l in BRAF+ vs 2.17 in BRAF− ($P=0.35$). The rate of central LNMx in patients undergoing PLNCD, was higher in BRAF+ than in BRAF− (63.6 vs 33%) ($P=0.37$). Thyroglobulin level before thyroid RAI ablation was 5.65 ng/ml in BRAF+ vs 3 ng/mL in BRAF− ($P=0.9$).
Conclusions
We did not find any significant association between BRAF+ and clinical and pathological features, not even with the presence of LNMx, probably due to the small size of the sample. Nevertheless, considering the high prevalence of occult LNMx in patients harbouring BRAF mutation, preoperative analyses of BRAF could possibly be helpful to decide initial surgery in patients affected of PTC, as it has been suggested previously.
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P1127

A case of papillary thyroid carcinoma in a patient with familial adenomatous polyposis of the colon
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Thyroid carcinoma is a rare extraintestinal manifestation of colonic familial adenomatous polyposis (FAP). This syndrome is caused by germline mutations in adenomatous polyposis coli (APC) gene, on chromosome 5q21-q22. Many patients manifest various extracolonic features: upper gastrointestinal adenomas, congenital hypertrophic retinal pigment epithelial lesions, desmoids tumors, and

thyroid, adrenal and brain cancers. It has been reported an incidence of 1.2% of thyroid carcinomas in FAP patients. We report a case of a female with this association.
Case presentation
A 43 year-old woman was seen for subclinical hypothyroidism. Her medical history was known for FAP. She underwent subtotal colectomy in 1995, and a colonic adenocarcinoma was found during pathologic study. Later genetics showed c.543–546del in APC. Several intestinal obstructions led to abdomino-perineal amputation and colostomy. In 2012, she required duodenoapancreatectomy since duodenal premalignant tumours were found. These surgeries had several postoperative complications and nowadays she suffers from short intestine syndrome with intermittent requirements of intravenous feeding. Also she had been operated for a desmoid tumor of abdominal wall and as teenager of posterior cranial fosa neuroblastoma that required chemo and radiotherapy.
Thyroid nodules were found in 2008 consulting for mild thyroid dysfunction. Neck exploration evidenced low grade goiter. Ultrasound imaging revealed multiple solid and cystic nodules. The biggest solid nodule and a calcified one were biopsied (large needle biopsy), results were not consistent with malignancy. Total thyroidectomy was proposed and performed in 2010. Pathologic description confirmed bilateral and multiple papillary carcinoma (no extrathyroidal extension). After that she underwent radioactive iodine ablation. Currently, the patient is treated with suppressive dose of levothyroxin and free of thyroid disease.
Conclusion
We present a case of a rare disease, FAP, with an extracolonic tumor, a papillary thyroid cancer. We asses the importance of early ultrasound exploration and surgical option if dubious results in these patients since the higher incidence of malignancy.

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P1128

Metastatic papillary thyroid carcinoma arising from thyroglossal duct cyst

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The incidence of thyroid papillary carcinoma in thyroglossal duct cyst is $<1\%$. Regional lymph node metastasis is usually seen in cases who have intrathyroidal tumor. We report a case of thyroid papillary carcinoma arising from thyroglossal duct cyst with lateral lymph node metastasis who have a normal thyroid gland and benign central compartment lymph nodes.
Case report
A 38-year-old male patient was admitted with the complaint of swelling in the neck. It was a cystic lesion (thyroglossal duct cyst?) and 16×32 mm in size on ultrasonography. The thyroid gland ultrasound was normal. There was a lesion on the left neck extending up from the upper to the lower jugular chain, measured 55×25 mm in size, containing millimetric calcifications which was suspected to be metastasis of papillary thyroid carcinoma. The fine needle aspiration cytology of this lesion was positive for metastases of papillary carcinoma of the thyroid. Thus, thyroglossal duct cyst excision and bilateral total thyroidectomy with central and left neck dissection was performed.
Thyroglossal duct cyst specimen pathology has been reported as an invasion of the bone and soft tissue in the form of papillary thyroid carcinoma. All of the central lymph nodes were reactive and thyroid gland was normal but there was a metastatic lymph node in the lateral neck dissection which is 7 cm in diameter.
Conclusion
This is a rare case due to have a thyroid papillary carcinoma arising from thyroglossal duct cyst with lateral lymph node metastasis and without a tumor in thyroid gland and without central lymph node metastasis. As a result, while evaluating a thyroglossal duct cyst, in all cases, a detailed neck ultrasonography should be performed and biopsy should be done from suspected lymph nodes before planning the surgery.

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P1129

Thyroid cancer frequency in acromegalic subjects

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Introduction
It is now well demonstrated that thyroid disorders are more frequent in acromegaly than in normal population. Our aim is to analyze thyroid cancer frequency in a cohort of Algerian subjects with acromegaly.
Material and methods
It is a retro and prospective study where 77 hyper somatotropic subjects were enrolled (34F and 43M) mean age = 39ans (12–58). Mean GH rate = 59 ng/ml (7–597, $n<5$). All had clinical examination, routine analyses, hormonal assessment, echosonography, fine nodule aspiration, and cytology. When the cytology was suspect or malignant the patients were operated on.
Results
Among this group, one subject (female case) had a well proved vesicular thyroid cancer (1.29%), and it was a multifocal one.
Conclusion
Our frequency, equal to 1.29%, is similar to Gasperi's multicenter study in Italy and lower than De Santos' work in Brazil (7.2%). Although thyroid cancer frequency varies from study to study, it is higher than in normal population. So, patients with acromegaly should be systematically submitted to thyroid ultrasounds. If nodules are found a fine nodule aspiration and cytology are recommended for all the nodules, especially when they are solid, hypo echoic, larges or with imprecise limits.

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P1130

Poor prognosis of recurrent and persistent papillary thyroid carcinoma with high anti-thyroglobulin antibody titers

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Background
In patients with papillary thyroid cancer (PTC), serum thyroglobulin (Tg) levels are monitored in order to detect disease recurrence. However, these patients may show anti-thyroglobulin antibodies (TgAb) in serum, which may influence the quantitation of Tg level and thus the confirmation of disease recurrence. The aim of this retrospective study was to show clinical outcome and the diagnostic strategy of recurrent or persistent PTC patients with high serum TgAb titer.
Subjects and methods
Details of 12 patients with recurrent or persistent PTC who had undergone total thyroidectomy and who had higher than normal TgAb titers at recurrence or during persistent disease were recorded in our thyroid cancer registry database. The clinical outcomes as well as the diagnostic approaches used to screen for cancer metastasis in these cases were reviewed.
Results
There were five women and seven men with a median age of 44 years old (range, 24–66 years). The median follow-up duration was 10.7 years (range, 0.3–21.6 years). Nine patients had recurrent PTC, and the other three had persistent disease. Five of the 12 patients had died of metastatic PTC during the follow-up period. Ten of the 12 patients had undetectable Tg levels at recurrence or during persistent disease. Thyroid ultrasonography is a reliable diagnostic tool for neck lymph node metastasis or local recurrence. Computed tomography revealed cancer metastases in ten patients, while I-131 scan failed to detect cancer metastases in three patients.
Conclusions
High serum TgAb titers in PTC patients at recurrence or during persistent disease may indicate disease progression and poor clinical outcome. Because serum Tg levels may fail as a surrogate tumor marker, a variety of imaging studies should be performed in order to screen for cancer metastasis and to initiate the aggressive treatment needed for these patients.

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P1131

Well differentiated thyroid-type carcinoma arising from struma ovarii a report of two cases

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We report two rare cases of well differentiated thyroid-type carcinoma arising from struma ovarii managed in our institution.
Case 1
A 41-year-old female admitted due to abdominal pain and vomiting. Her abdomen was globular with palpable vague mass on lower abdomen. Ultrasound scan revealed 12.5×12.1×8.3 cm heterogenous mass with ascites. Total abdominal hysterectomy with bilateral salphingo-oophorectomy was done and histopathology results revealed papillary thyroid-type carcinoma arising from struma ovarii. Total thyroidectomy, radioactive iodine ablation and thyroxine suppression therapy were done. On follow-up there was no evidence of persistent tumor or recurrences.
Case 2
A case of 38-year-old, G3P2, female diagnosed with 16×15×12 cm cystic ovarian mass during the second trimester of her third pregnancy, underwent right salphingo-oophorectomy due to persistent abdominal pain. Histopathology revealed follicular carcinoma of thyroid type with no metastases. Further treatment of the malignancy was delayed due to current pregnancy. Patient delivered a healthy appropriate for gestational age baby boy via a spontaneous vaginal delivery. She is now scheduled to undergo thyroidectomy, radioactive ablationa and thyroxine suppressive therapy.
Due to the rarity of the disease, protracted clinical course and lack of uniform histological criteria for malignancy, diagnosis and management are still not universally accepted. Recent recommendations showed that well differentiated thyroid-type carcinoma arising from stuma ovarii as presented in our two cases should be diagnosed and managed as primary well differentiated carcinoma of thyroid gland.

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P1132

Male patient with neck mass: ectopic cancer vs metastasic disease

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Case of 33 years old male patient with an unremarkable past medical history, who after a car accident, a cystic nodular right neck mass was incidentally found by MRI of cervical spine. Physical examination revealed a mildly tender right neck mass. He was clinically and biochemically euthyroid. Denied history of neck irradiation. No family history of thyroid disease or thyroid cancer. Excisional biopsy of right neck mass showed metastasic well differentiated papillary carcinoma of thyroid gland at lymph node. Total thyroidectomy with right modified neck dissection was performed. Histopathological report disclosed no evidence of malignant tumor within the thyroid gland (papillary cancer) as well as 16 right lymph nodes obtained from surgery. One month after surgery, thyroglobulin levels were elevated, 133.61 ng/ml (nv: 5–25 ng/ml) with negative anti-thyroglobulin antibodies (thyroglobulin antibodies: 11.8 μU/ml nv: 0–4.11). Thyroid scan and whole-body scan showed evidence of functional thyroid tissue remnants; this appears to be a right thyroid lobe. Also, a faint visualization of a smaller focus in the left thyroid bed region was seen. Thyroid ultrasound showed two right medial neck nodules. Fine needle aspiration biopsy of neck nodules were reported positive for metastasic thyroid papillary carcinoma. Radioidine ablation therapy was given.
Ectopic thyroid gland is defined as thyroid tissue not located anterolaterally in the second to fourth tracheal cartilages. Ectopic Thyroid tissue is a rare entity with an incidence of 1 in 300.000. An ectopic thyroid gland in the region of the submandibular gland, intra-trachea or laterally is very rare. Malignant transformation of ectopic thyroid tissue is an uncommon event; only 43 cases have been reported. Only 10 of those cases were papillary carcinoma.

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P1133

Papillary carcinoma with diffuse toxic guatr and highly resistant course

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Introduction
The coexistence of hyperthyroidism and thyroid cancer is considered a rare event. We describe a case of papillary carcinoma of the thyroid that mimicked a diffuse toxic goiter.
Case report
A 26-year-old female patient presented with a complaint of malaise was diagnosed to have thyrotoxicosis and following the tests performed (sT_3: 7.7 (1.7–3.7 ng/dl), sT_4: 2.5 (0.7–1.49 ng/dl) TSH: <0.0025 (0.35–4.94 μU/ml)). Patient was clinically stable; without any palpitation, sweating, weight loss or ophthalmopathy. Thyroid was palpable and graded 1b according the Physical examination. Ultrasonographic findings were as follows: both lobes were heterogeneous, hypoechoic lobulated and no nodule was identified. Scintigraphic evaluation was not performed due to a problem at thyroid scintigraphy device. Anti-thyroglobulin antibody, anti-TPO antibody and TSH receptor antibody were found to be negative. The patient was initially started on methimazole and due to development of rash she was then switched to propylthiouracil (PTU). Antithyroid treatment given for 4 months with PTU 3×200 mg and propranolol 4×40 mg did not improve thyrotoxicosis therefore relevant surgical operation was taken into consideration. Although the patient was given PTU 3×200 mg, lithium 3×400 mg (pre-operatively for 1 month), lugol solution 3×5 drops (pre-operatively for 10 days), prednol 3×20 mg (pre-operatively for 5 days) orally for pre-operative preparation, the patient's status of thyrotoxicosis persisted and she was operated in spite of the existing risk. During and after the operation, no complications were encountered and the patient's pathology result was found to be consistent the background of diffuse inflammation with areas of papillary thyroid carcinoma.
Conclusions
This is an interesting case that manifests a highly resistant course and has a coexistence of hyperthyroidism and thyroid cancer.

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P1134

Brain metastases of papillary thyroid cancer: a case report and literature review

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Introduction
Papillary thyroid carcinoma is characterized by a good prognosis and a slow progression. Its metastases are usually located in cervical lymph nodes. But, sometimes they can reach the lung and bones. Cerebral metastases are exceptional as they have been reported in only 4%. Single or multiple, they can be totally symptomatic, but they are life threatening and require specific care as in the following case
Case report
A man aged 56, was referred to our unit for vertebral metastasis whose biopsy argued for thyroid origin. Histological examination of the thyroid confirmed the papillary form which was classified as T3NxM1. He previously underwent radiotherapy for compression of the spinal cord. Radiological explorations demonstrated lung and bone metastases. Thyroglobulin rates were very high (>600 ng/ml). Just before radio iodine intake, he suddenly suffered from vomiting and headaches evocating increased intracranial pressure. Brain CT scan showed multiple brain lesions with hydrocephaly needing a ventricular shunt. Unfortunately just before being operated on, he died from severe cerebral haemorrhage.
Conclusion
Brain metastases are very rare in papillary thyroid carcinoma. They are usually seen in the late stage of the disease as in our case. An early detection and a specific treatment can probably improve the quality of life and avoid brain hypertension and cerebral haemorrhage.

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Choroidal bilateral metastases from thyroid carcinoma: report of a case and review of the literature

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Introduction
Metastases of thyroid carcinoma to the choroid and/or orbit are rare and usually occur at an advanced stage of the disease. The principal symptoms are decreased or blurred vision and/or persistent eye pain but they can be totally asymptomatic. They are diagnosed by ultrasound ocular CT and/or MRI scanning. The diagnosis is certain if they fix radioactive iodine. Their prognosis is very bad. Enucleation is the treatment of choice in cases of visual impairment. We report a case and will review the data in the literature.
Observation
MH 58-year-old woman treated for thyroid carcinoma with multiple secondary metastases (lung, bone, adrenal, and liver). Biopsy of a pulmonary localization was in favor of a primitive thyroid origin. Ophthalmological examination after a bone localization in the roof of the orbit showed painless palpable mass in the left orbital arch, reduced vision of the right eye. Angiography, ultrasound b, and OCT were in favor of bilateral choroidal metastases.
Conclusion
Choroidal metastases of thyroid carcinomas are rare and occur in the context of metastatic seeding. The diagnosis is made through non-invasive imaging methods. The therapeutic management is difficult because secondary lesions of thyroid origin are resistant to chemotherapy and radiotherapy.

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Thyroglobulin measurement in fine needle aspirates from cervical lymph nodes

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Introduction
Thyroglobulin (TG) measurement in fine needle aspirates cytology (FNAC) needle washout has become an important method for diagnostic of cervical lymph nodes, particularly in patients with differentiated thyroid cancers, but also in some patients before the diagnostic. We report some clinical cases to confirm its importance in these situations.
Patients and methods
In our department we use this method since 2007. We report seven patients with thyroid disease referred to our department. The ultrasound examination revealed the presence of suspicious cervical lymph nodes. FNAC was performed in the thyroid nodules. The lymph nodes were punction for cytology, together with the TG in the needle washout in the last.
Results
From the patients with high TG, four were already submitted to thyroidectomy, confirming the diagnostic of papillary cancer. Patient 5 has TG antibody elevated, which can contribute to the low level of TG.

Pts	Lymph node citology	TG (needle)	Nodule cytology	Gender	Age	Previous cytology
1	Th. tumour?	24 837 ng/ml	Insufficient	Female	65	Insuf
2	Insufficient	250 219	Papillary	Female	36	Coloid
3	Insufficient	15 999	Coloid	Female	43	Coloid
4	Insufficient	12 259	Coloid	Female	34	Coloid
5	Papillary m	48	–	Male	19	Insuf
6	Lymphoma	<0.2	Insufficient	Male	36	–
7	Lymphoma	<0.2	–	Male	28	–

Conclusion
In these patients, TG in the needle washout in FNAC proved to be a good contribution to the diagnostic of malignancy, making its treatment faster and more efficient. The role of this method is particularly relevant due to the high rate of 'insufficiency' in the cytology of lymph nodes (cystic).

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Poorly differentiated thyroid carcinoma: a clinicopathologic study of 30 cases

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Introduction
Poorly differentiated thyroid carcinoma are considered as a link between well differentiated carcinoma and undifferentiated ones. The histological definition is still controversial and various criteria have been proposed.
Objective
The main objective of this study is to assess the management of poorly differentiated thyroid cancer in our department.
Materials and methods
We reported 30 patients with poorly differentiated carcinoma diagnosed during the period of 5 (05) years (2008–2012).
We analysed the management of these patients. There were classified in age, sex, clinical outcome, initial distant metastasis, treatment (surgery, radiotherapy, and chemotherapy) and evolution.
Results
A 30 patients diagnosed between January 2008 and December 2012.
Female in majority.
Middle age 45 years (25–72), 75% with distant metastasis at diagnosis (brain, bone and pulmonary).
Surgery was the first treatment proposed: total thyroidectomy practiced in 60%; in other cases partial thyroidectomy or biopsy due to local tumor invasion.
Radioiodine therapy was indicated after surgery, not possible to be done in 10 patients because of aggressiveness.
Radiotherapy or external beam irradiation when incomplete surgery.
Chemotherapy done for two patients.
Evolution: death was reported in seven patients, stabilization performed in ten patients even with distant metastasis. Disease progression in 13 patients.
Discussion
Poorly differentiated thyroid cancers are defined as follicular neoplasm with intermediate position. There was The Turin proposal (2006): the first criteria were solid, trabecular or insular growth, they add presence of necrosis, high mitotic index and nuclear features.
The histology is the first difficulty of this entity.
The aggressiveness of this pattern need an early management and treatment. surgery and radio iodine therapy seem to give the best results when possible. Other treatment are done when disease progression with distant metastasis.
The prognosis of this entity is very bad especially when distant metastasis are at diagnosis.

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Adrenal metastases from thyroid cancers

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Introduction
Adrenal metastases due to thyroid cancers deemed to very rare as adrenal glands are not a common site of metastases from thyroid cancer. When the phenomenon occurs the prognosis is very poor as adrenal metastases usually do not fix radioactive iodine. Our aim is to report three cases.
Case reports
No. 1: a woman aged 57 harbouring anaplastic thyroid cancer classified T4, N1, M0, consulted for back pain, severe fatigue and skin pigmentation. Abdomen ultrasound showed very large tumours in both adrenals. Hormonal assessment demonstrated low cortisol with high ACTH. Although she was taking glucocorticoids at high dose, she died 1 month later after an adrenal crisis.
No. 2: a woman aged 56 was referred for oncocytic papillary carcinoma with pulmonary and bone metastases. Checking for other sites showed liver and adrenal localizations. Fine nodule aspiration of large adrenal masses confirmed the thyroid origin. More than 1 year later she is still alive, but with a large diffusion of the disease.

No. 3: a 53-year-old woman was sent for a suspect thyroid tumour. Body scan showed pulmonary, lymph nodes and bones metastases. After surgery the papillary thyroid cancer was confirmed. Some months later a right adrenal mass was discovered. She died 2 years later.

Conclusion

The 3 three women aged 53–57 have adrenal metastases from thyroid cancers. It was an anaplastic form in one case, and a papillary aggressive form in two other cases. Life duration varies from 1 month to 2 years after the adrenal metastases were diagnosed. Endocrinologist should check systematically for those metastases and trait them by surgery if they are at an early stage, if not the prognosis is poor as in our cases.

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Thyroid papillary carcinoma in a toxic adenoma: case report

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Introduction

Malignant toxic thyroid nodules are rare, with few cases described in the literature. The prevalence of malignancy in toxic adenomas varies between 1 and 8% in different series (most, corresponding to $<2.5\%$). For that reason, international guidelines recommend that they should not be submitted to fine needle aspiration biopsy.

Case report

A 42-year-old female patient without relevant previous medical history was admitted to our hospital because of two thyroid nodules (37 e 13 mm) in the left lobe (without adenopathies); analytically, she had subclinic hyperthyroidism. Fine needle aspiration biopsy diagnosis was colloid nodular hyperplasia; ^{131}I scan showed tracer activity in a left nodule with suppression of extranodular tissue. Thyroid hormone measurements were repeated and were compatible with clinical hyperthyroidism. She was initially treated with methimazole and then submitted to left hemithyroidectomy which showed a thyroid papillary carcinoma (folicular variant) in both nodules associated with a lymph node metastasis. She was finally submitted to total thyroidectomy (remaining tissue didn't present neoplastic involvement) and ^{131}I ablative treatment and remains under surveillance with levothyroxine suppressive therapy.

Conclusions

The authors describe a rare association of hot nodules and papillary thyroid carcinoma, with a nondiagnostic cytology, stressing the importance of long-term surveillance of toxic nodules that underwent nonsurgical therapies. Published cases of differentiated thyroid carcinoma appearing in hot nodules seem to suggest a similar prognosis to that present in nontoxic nodules.

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Ultrasonographic signs suggestive of malignancy in thyroid nodules

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Introduction

Thyroid ultrasound is a widely used tool in monitoring thyroid nodule, allowing us to assess the size and other sonographic signs suspicious of malignancy, facilitating decisions about diagnosis and treatment.

Objectives

To evaluate the presence of ultrasonographic signs suggestive of malignancy in patients with thyroid carcinoma in our hospital.

Material and methods

Retrospective study of 63 patients with thyroid carcinoma in our hospital (2009–2011). We analyzed: age, sex, sonographic features (ECO held during the year before surgery) and pathological definitive diagnosis.

Results

We analyzed 63 patients, 54 were women (83.1%) with mean age of 49.23 ± 15.48 years. The final pathologic diagnosis was: papillary thyroid carcinoma: 84.6%, follicular thyroid carcinoma: 10.8%, medullary carcinoma: 1.5% and others 3.1%.

Previous ultrasound data

41 (63.1%) were single nodule, the rest were multinodular thyroid goitier. The average size of the nodule was 27.6 ± 16.5 mm. 50 nodules (76.9%), were solid, the rest were solid-cystic. Hypoechogenicity was reported in 19 (30.6%) (no record of echogenicity 58%), calcifications in 13 (20.6%), central intranodular vascularization in 10 (15.9%), irregular margins in 5 (7.7%) peripheral halo formation in 9 (14.3%) and presence of regional lymphadenopathy in 9 (15%). With respect to volume, three-dimensional sonographic size was reported in 29.5%, two in 26.2% and only one in 44.3%.

We detected at least one ultrasound data suggestive of malignancy in 42.8%, two in 31.7%, three in 11.1% and four in 7.9%. These data were not detected in 6.3% (4 patients).

Conclusion

Our results confirm that thyroid ultrasound can help in the management of thyroid carcinoma because most patients had any signs of malignancy.

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