Third EASD Incretin Study Group Meeting

24 - 26 January 2019 · Bochum, Germany

Venue: Lecture Hall, St. Josef Hospital,
Ruhr-University Bochum, Gudrunstraße 56,
D-44791 Bochum, Germany

Scientific organization:

Prof. Dr. Michael A. Nauck (chairman) for the
Steering Committee of the EASD Incretin Study Group

Laura Kupfer (assistant)

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Ausrichter:

Katholisches Klinikum Bochum gGmbH

EASD

Incretin Study Group

Internet appearance:
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Steering committee

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Organisational information

Venue: **Lecture Hall, St. Josef-Hospital (Ruhr-University Bochum)**
Gudrunstraße 56, D-44791 Bochum, Germany

Hotels:

H+Hotel,
Stadionring 22, 44791 Bochum
Check-in from: 15:00, Check-out until: 12:00

Renaissance Bochum Hotel,
Stadionring 18, 44791 Bochum
Check-in from: 16:00, Check-out until: 12:00

Courtyard Marriott Bochum Stadtpark Hotel,
Klinikstr. 43-45, 44791 Bochum
Check-in from: 15:00, Check-out until: 12:00

All hotels are in walking distance from the conference venue.

Airport:

Düsseldorf (train station Düsseldorf-Flughafen;
45 min to Bochum Hauptbahnhof)

Train station:

Bochum Hauptbahnhof

Local train:

(from Bochum Hauptbahnhof),
306 or 308 (direction Schürbank-Straße)

to

Ruhrstadion (Castroper Straße),
closer to H+ and Renaissance hotels

or to

Planetarium (Castroper Straße),
closer to Marriot Courtyard hotel

Taxi: **(costs approximately 10 €)**

Day 1 – Thursday 24th Jan 2019

14:00 - 17:00 **Registration**

Scientific program

Day 1 – Thursday 24th Jan 2019

17:00 - 17:15 **Opening remarks**
Michael A. Nauck, Bochum, Germany

17:30 - 18:30 **Opening Session:**
Non-canonical expression of prohormone convertases and "aberrant" post-translational processing in the endocrine pancreas and gut: Evidence from animal and human examinations and potential physiological importance

Chairman: **Wolfgang E. Schmidt**, Bochum, Germany

Piero Marchetti, Pisa, Italy:
GLP-1 and GIP production in pancreatic islets
(20 min talk, 10 min discussion)

Filip Knop, Copenhagen, Denmark:
"Pancreatic-type" glucagon from intestinal L cells
(20 min talk, 10 min discussion)

19:00 ***Buffet Dinner***
(Lobby of the Lecture Hall)

Day 2 – Friday 25th Jan 2019

08:30 - 10:30 Basic research - **Secretion and actions of GIP and GLP-1**

Chairman: **Remy Burcelin, Toulouse, France**

No.	Presenter	Title	From	To
OP 1	Ämmälä, C. (AstraZeneca, Mölndal, Sweden)	GLP1 peptide as a vehicle for targeted delivery of antisense oligonucleotides to pancreatic ß-cells	8:30 AM	8:40 AM
OP 2	Sachs, S. (Helmholtz Zentrum München, Munich, Germany)	Glucagon-like peptide 1 targeted delivery of estrogen redifferentiates functional β-cell mass in diabetes	8:45 AM	8:55 AM
OP 3	Bitsi, S. (Imperial College London, London, United Kingdom)	Agonist-induced palmitoylation of the GLP-1R regulates receptor recruitment to the lipid rafts, internalisation and signalling	9:00 AM	9:10 AM
OP 4	Brierley, D.I. (UCL, London, United Kingdom)	Potentiation of satiation by glucagon-like peptide-1 producing preproglucagon neurons	9:15 AM	9:25 AM
OP 5	Pickford, P. J. (Imperial College, London, UK)	Functional effects of biased oxyntomodulin analogues	9:30 AM	9:40 AM
OP 6	Lu, V. (University of Cambridge, Cambridge, United Kingdom)	Adenosine triphosphate (ATP) is released from glucagon-like peptide 1 (GLP-1) secreting cells and signals to vagal afferent neurons	9:45 AM	9:55 AM
OP 7	Roell, W. (Eli Lilly and Company, Indianapolis, United States)	GIP signaling integrates with insulin signaling to regulate glucose and lipid metabolism in human adipocytes	10:00 AM	10:10 AM
OP 8	Larraufie, P. (WT-MRC Institute of Metabolic Science, Cambridge, UK)	Peptidomics to study enteroendocrine cells after bariatric surgery	10:15 AM	10:25 AM

10 min presentation and 5 min discussion

10:30 - 11:00 **Break**

Day 2 – Friday 25th Jan 2019 (continued)

11:00 - 12:30 Basic research - **GLP-1, GLP-1 metabolites, bihormonal receptor agonists, and glucagon**

Chairman: **Jens J. Holst, Copenhagen, Denmark**

No.	Presenter	Title	From	To
OP 9	Jepsen, SL. (Department of Biomedical Sciences and NNF Centre for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark)	Blockade of the somatostatin receptor subtype 2 and 5 increases GLP-1 secretion, resulting in GLP-1 receptor-mediated lowering of blood glucose in mice	11:00 AM	11:10 AM
OP 10	Burcelin, Remy. (Inserm, Toulouse, France)	Liraglutide controls insulin secretion via the enteric nervous system through vagus nerve to beta cell axis	11:15 AM	11:25 AM
OP 11	Picard, A. (Center for Integrative Genomics, Lausanne, Switzerland)	A genetic screen identified hypothalamic IL-1R signaling as a regulator of insulin-induced glucagon secretion	11:30 AM	11:40 AM
OP 12	Ramracheya, R. (Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, UK)	Elucidating the effect of the GLP-1 metabolite, GLP-1(9-36) on glucagon secretion in mouse and human islets	11:45 AM	11:55 AM
OP 13	Grasset, E. (I2MC, Toulouse, France)	Gut microbiota dysbiosis of type 2 diabetic mice impairs the intestinal circadian rhythm of GLP-1 sensitivity	12:00 PM	12:10 PM
OP 14	Roell, WC. (Eli Lilly and Company, Indianapolis, USA)	LY3298176, a novel dual GLP-1/GIP receptor agonist improves weight loss and glycemic control in preclinical models compared to selective GLP-1R agonist	12:15 PM	12:25 PM

10 min presentation and 5 min discussion

12:30 - 13:30 **Lunch**

Day 2 – Friday 25th Jan 2019 (continued)

13:30 – 15:30 Clinical research – **GIP, GLP-1 and glucagon acting alone or in combination**

Chairman: **Filip Knop, Copenhagen, Denmark**

No.	Presenter	Title	From	To
OP 15	Stensen, S. (Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark)	Glucose-dependent insulinotropic polypeptide receptor antagonism in patients with type 2 diabetes	1:30 PM	1:40 PM
OP 16	Gasbjerg, L. S. (Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark)	Separate and combined metabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals: Two clinical studies	1:45 PM	1:55 PM
OP 17	Daniele, G. (Dept. of Clinical and Experimental Medicine, University of Pisa, Italy)	Impaired brain plasticity in obesity: the effects of bariatric surgery and gut hormones	2:00 PM	2:10 PM
OP 18	Alexiadou, K. (Imperial College, London, UK)	Gut hormone changes post Roux-en-Y gastric bypass: One year prospective study	2:15 PM	2:25 PM
OP 19	Jermutus, L. (MedImmune, Cambridge, UK)	Effects of MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, on pancreatic and incretin hormones	2:30 PM	2:40 PM
OP 20	Parker, V. E. R. (MedImmune, Cambridge, UK)	MEDI0382, a dual GLP-1 glucagon receptor agonist, promotes rapid glucose control and significant weight loss in patients with type 2 diabetes	2:45 PM	2:55 PM
OP 21	Haupt, A. (Eli Lilly and Company, Indianapolis, USA)	LY3298176, a novel GIP and GLP-1 receptor dual agonist for the treatment of type 2 diabetes mellitus: First human dose to proof of concept in a randomised Phase 1b clinical trial	3:00 PM	3:10 PM
OP 22	Frias, JP. (National Research Institute, Los Angeles, USA)	Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial	3:15 PM	3:25 PM

10 min presentation and 5 min discussion

Day 2 – Friday 25th Jan 2019 (continued)

15:30 - 16:00 **Break**

16:00 - 17:15 **Highlight Lecture –**
Michael Horowitz, Adelaide Australia:
Gastric emptying, secretion of gastrointestinal peptides and insulin, and glucose homeostasis
Chairman: **Juris J. Meier, Bochum, Germany**
45 min talk + 30 min discussion

17:30 Transfer to **Vfl Bochum Soccer (Vonovia Ruhr-)Stadium, Stadtwerke Bochum Lounge**,
Castroper Strasse 145, 44791 Bochum
(a 10 min walk across the street)

Cocktails will be served before and during the poster sessions

18:00 - 19:00 Poster sessions 1, 2, 3 and 6

19:00 - 20:00 Poster sessions 4, 5, 7 and 8

Day 2 – Friday 25th Jan 2019 – Poster Sessions

18:00-18:45 **Poster Session 1** Basic science **– GLP-1 and GIP secretion and biological effects**

Chairwoman: **Fiona Gribble, Cambridge, United Kingdom**

No.	Presenter	Title
PO 1	Vana, V. (Department of Drug Design and Pharmacology, University of Copenhagen, Denmark)	Gut hormone plasma levels after voluntary fat intake in a mouse self-administration model
PO 2	Yang, M. (Wellcome Trust-MRC Institute of Metabolic Science, Cambridge, United Kingdom)	Amino acid sensing in K and L cells from mouse duodenal organoids
PO 3	Christiansen, C. (NNF Center for Basic Metabolic Research & Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark)	Mechanisms of bile acid-induced glucagon-like-peptide 1 and peptide-YY secretion from the colon
PO 4	Hunt, J. (Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark and NNF Center for Basic Metabolic Research, University of Copenhagen, Denmark)	Dietary fiber is essential to maintain intestinal weight and L cell secretion
PO 5	Montaner, M. (CNRS Université Paris Diderot, Paris, France)	Acute injection of the GLP-1 analog Exendin-4 in the olfactory bulb improves glucose tolerance in diet-induced obese mice.
PO 6	Adriaenssens, A. (Institute of Metabolic Science, University of Cambridge, UK)	The role of glucose-dependent insulinotropic polypeptide receptor-expressing neurons in the hypothalamus
PO 7	Gault, VA. (University of Ulster)	A GIP/GLP-1 hybrid peptide exhibits beneficial actions on hippocampal function in dietary-induced diabetic mice
PO 8	Naughton, V. (Ulster University, Coleraine, NI, United Kingdom)	Roux-en-Y gastric bypass has major effects on jejunal proteomics of Zucker diabetic fatty rats
PO 9	Flatt, P.R. (Ulster University, Coleraine, United Kingdom)	Beneficial effects of GLP-1 receptor activation on metabolic control and beta-to-alpha cell transdifferentiation in multiple low-dose streptozotocin diabetes

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

18:00-18:45 **Poster Session 2** Basic Science – **Identification of entero-endocrine cells and their mode of secretion**

Chairman: **Peter Flatt, Londonderry, North Ireland, United Kingdom**

No.	Presenter	Title
PO 10	Goldspink, DA. (Institute of Metabolic Science, Cambridge, UK)	Modelling human enteroendocrine cells using adult intestinal organoids
PO 11	Darwish, T. (University of Cambridge, Wellcome Trust-MRC Institute of Metabolic Science, Cambridge, UK)	Neutral amino acid transporter: SLC6a19- linking amino acid sensing to gut hormone secretion?
PO 12	Andersen, DB. (University of Copenhagen, Copenhagen, Denmark)	Systematic mapping of tissue expression of glucagon-like peptide 1 receptor using an unbiased knock-in mouse model
PO 13	Khan, D. (Ulster University, Coleraine, United Kingdom)	Alterations of enteroendocrine L- and K-cells populations with insulin deficiency and insulin resistance
PO 14	Modvig, I.M. (University of Copenhagen, Copenhagen, Denmark)	Peptone mediated glucagon-like peptide 1 secretion depends on intestinal absorption and activation of basolaterally located Calcium-Sensing Receptor
PO 15	Miedzybrodzka, E. L. (Metabolic Research Laboratories, University of Cambridge, United Kingdom)	The contribution of TRP channels to L-cell signalling
PO 16	Smith, C. (Institute of Metabolic Science, University of Cambridge, UK)	Tracking the development and release of hormone-containing vesicles in murine L-cells
PO 17	Reimann, F. (University of Cambridge, Cambridge, UK)	Classification of colonic enteroendocrine cells based on single cell mRNA expression analysis

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

18:00-18:45 **Poster Session 3** Basic science – **Gut-derived peptides and glucagon**

Chairman: **Benard Thorens, Lausanne, Switzerland**

No.	Presenter	Title
PO 18	Kay, R. G. (Metabolic Research Laboratories, University of Cambridge, UK)	Proglucagon peptide analysis – will the real peptide please stand up!
PO 19	Foreman, R. (Metabolic Research Laboratories, University of Cambridge, Cambridge, UK)	A multiplex method for quantifying gut hormone peptides using Mass Spectrometry
PO 20	Galvin, S. (Institute of Metabolic Sciences, Cambridge, UK)	Characterisation of novel gut-derived peptides in vivo and in vitro
PO 21	Marrano, N. (Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy)	Can irisin be considered an incretin-like hormone?
PO 22	Hunt, J. (Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark and NNF Center for Basic Metabolic Research, University of Copenhagen, DK)	Increased L-cell secretion in Gcgr-/- mice does not potentiate tumor growth
PO 23	Trabucco, M. (University of Florence, Florence, Italy)	The effects of glucagon on human adipose precursors
PO 24	Galsgaard, K.D. (Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, DK)	The amino acid citrulline does not increase blood glucose concentrations in glucagon receptor knockout mice
PO 25	Eriksson, O. (Antaros Medical AB, Mölndal, Sweden and Uppsala University, Uppsala, Sweden)	First-in-class PET tracer for the Glucagon receptor
PO 26	Woodward, ORM. (Institute of Metabolic Science, Cambridge, UK)	Distribution of G protein coupled relaxin/insulin-like family peptide receptor-4 (RXFP4) expressing cells in the mouse brain

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

19:00-19:45 **Poster Session 4** Basic science – **Novel aspects of incretin secretion and action**

Chairman: **Frank Reimann, Cambridge, United Kingdom**

No.	Presenter	Title
PO 27	Lewis, JE. (Institute of Metabolic Sciences, Cambridge, UK)	RXFP4, the cognate receptor for insulin-like peptide 5, alters food preference in mice
PO 28	Lucey, M. A. (Imperial College London, London, United Kingdom)	Anorectic effects of biased GLP-1 receptor agonists differ with central versus peripheral administration
PO 29	Liang, L. (Medimmune, Cambridge, United Kingdom)	Novel anti-obesity treatment by targeting glucagon-like peptide-1 and peptide-YY in diet-induced obese mice
PO 30	Trapp, S. (UCL, London, United Kingdom)	Variations in extracellular glucose concentration elicit intracellular Ca2+ changes in GLP-1 producing preproglucagon neurons in vitro
PO 31	Polex-Wolf, J. (Novo Nordisk, Maaloev, Denmark)	The GLP-1 receptor agonist semaglutide lowers body weight by direct activation of hypothalamic and hindbrain mechanisms
PO 32	Lyu, Z. (Imperial College London, London, UK)	Differential regulation of endocytic trafficking determines spatial and temporal organization of signaling from beta cell incretin receptors
PO 33	Smit, F.X. (University of Copenhagen, Copenhagen, Denmark)	Disclosing incretin receptor activation using molecular dynamics simulations
PO 34	Pacini, G. (Metabolic Unit, IN-CNR, Padova, Italy)	GIP and GLP-1 increase glucose effectiveness in normal and hi-fat fed mice regardless of changes in insulin resistance
PO 35	Lehrke, M. (UK-Aachen, Aachen, Germany)	Myocardial infarction is sufficient to increase GLP-1 secretion leading to improved left ventricular contractility and mitochondrial respiratory capacity

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

19:00-19:45 **Poster Session 5** Basic science – **Incretins and liver, bone, kidney, and lungs**

Chairman: **Richard Pratley, Orlando, FL, USA**

No.	Presenter	Title
PO 36	Janah, L. (Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, Univ. of Copenhagen, Copenhagen, DK)	Impaired hepatic glucagon signaling as a cause for non-alcoholic fatty liver disease
PO 37	Billeschou, A. (Department of Biomedical Sciences, Faculty of Health and Medical Sciences, Univ. of Copenhagen, DK)	The Glucagon-like peptide 2 receptor knock-out mouse and intestinal injury
PO 38	Kissow, H. (NNF Center of Basic Metabolic Res. and Dep. of Biomedical Sciences, Univ. of Copenhagen, Copenhagen, DK)	The link between GLP-1 and ANP explored in ex vivo perfused pig organs
PO 39	Balk-Møller, E. (NNF Center for Basic Metabolic Research and Dep. of Biomedical Sciences, Univ. of Copenhagen, DK)	Liraglutide treatment increases expression of atrial natriuretic peptide and decreases endothelin-1 expression in a mouse model of obstructive pulmonary disease
PO 40	Mabilleau, G. (GEROM - University of Angers, Angers, France)	The GLP-1 receptor agonist exenatide ameliorates bone composition and tissue material properties in high fat fed diabetic mice
PO 41	Gobron, B. (Groupe études remodelage osseux et biomatériaux, GEROM, CHU d'Angers, Angers, France)	Bone remodeling is tightly controlled by enteroendocrine K-cell products
PO 42	Möllmann, J. (UK-Aachen, Aachen, Germany)	Glucagon-like peptide-1 and its cleavage products are renoprotective in murine diabetic nephropathy
PO 43	Lærke, M. (Copenhagen University, Copenhagen, DK)	The fat sensing receptors GPR40 and GPR119 are decreased in the small intestine of mice after fat-self-administration
PO 44	Richards, P. (Kallyope Inc., New York, USA)	Establishing the cellular makeup of the gut-brain axis by single-cell sequencing

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

18:00-18:45 **Poster Session 6** Clinical science – **Gut, bile, and bihormonal receptor agonists in the therapy of diabetes and obesity**

Chairman: **Juan Frias, Los Angeles, CA, USA**

No.	Presenter	Title
PO 45	Murahovshi, V. (Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany)	Evidence of GIP-induced regulation of fatty acid desaturase 2 (FADS2) gene expression in subcutaneous adipose tissue
PO 46	Pratley, R. (Florida Hospital Translational Research Institute, Orlando, USA)	Effects of pioglitazone on glucose dependent insulinotropic polypeptide mediated insulin secretion and adipocyte receptor expression in patients with type 2 diabetes
PO 47	Pereira, S.S. (Unit for Multidisciplinary Research in Biomedicine & Dep. of Anatomy, Institute of Biomedical Sciences Abel Salazar, Univ. of Porto, Porto, Portugal)	Biliopancreatic diversion with duodenal switch (BPD-DS) and single-anastomosis duodeno-ileal bypass with sleeve gastrectomy (SADI-S) result in distinct post-prandial hormone profiles
PO 48	Mezza, T. (Fondazione Policlinico Universitario A. Gemelli IRCSS-Università Cattolica del Sacro Cuore, Roma, Italia)	Bile modulates secretion of incretins and insulin: a study on human extrahepatic cholestasis
PO 49	Monteiro, M.P. (Unit for Multidisciplinary Research in Biomedicine & Dep. of Anatomy, Institute of Biomedical Sciences Abel Salazar, Univ. of Porto, Porto, Portugal)	Disclosing gut dynamics eliciting post-bariatric hypoglycemia
PO 50	Guida, C. (Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, UK)	PYY plays a key role in the resolution of diabetes following bariatric surgery in humans
PO 51	Robertson, D. (MedImmune, Cambridge, UK)	Continuous glucose monitoring reveals comprehensive glucose control with MEDI0382 in patients with type 2 diabetes mellitus
PO 52	Ambery, P. D. (MedImmune, Cambridge, UK)	MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, significantly reduces hepatic fat content in subjects with type 2 diabetes mellitus
PO 53	Harger, A. (Institute for Diabetes and Obesity, Helmholtz Zentrum München, Munich, Germany)	GLP-1-mediated delivery of thyroid hormone T3 reverses diet-induced obesity and glucose intolerance in mice

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

19:00-19:45 **Poster Session 7** Clinical science – **GLP-1 receptor agonists and glucagon**

Chairwoman: **Tina Vilsbøll, Copenhagen, Denmark**

No.	Presenter	Title
PO 54	Bugliani, M. (University of Pisa, Pisa, Italy)	Effects of GLP-1R agonists on beta cell survival, function and granule motility
PO 55	Juhl, CR. and Jensen SBK. (Department of Biomedical Sciences and NNF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark)	Combination of GLP-1 receptor agonist (GLP-1RA) treatment and physical activity for maintenance of diet-induced weight loss and metabolic health
PO 56	Juel, CTB. (Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark)	The glucagon-like peptide-1 receptor agonist lixisenatide reduces postprandial glucose excursions in totally pancreatectomised patients
PO 57	Henningson Johnson, F. (Mercodia AB, Uppsala Sweden)	Levels of GIP, GLP-1, insulin and glucagon in type 2 diabetes and healthy subjects after a mixed meal tolerance test
PO 58	Farngren, J. (Department of Clinical Sciences Lund, Lund University, Sweden)	Incretin-based treatment: Glucose threshold for glucagon counter-regulation during hypoglycemia
PO 59	Juel, CTB. (Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark)	Intravenous arginine has no effect on the secretion of gut-derived glucagon in totally pancreatectomized subjects
PO 60	Stojanovska, Elena. (University of Copenhagen, Copenhagen, Denmark)	Intestinal glucagon and its role in diabetic hyperglycaemia
PO 61	Alsalim, W. (Dept. of Clinical Sciences Lund, Lund University, Lund, Sweden)	Comparing the effect of three different DPP-4 inhibitors during 24 hours Cross-over study in metformin-treated type 2 diabetes individuals
PO 62	Hoelscher, C. (Copenhagen University, Neuroscience Dept., Copenhagen, Denmark)	Incretin mimetics show neuroprotective effects in neurological disorders

6 min per poster (3 min presentation and 3 min discussion)

Day 2 – Friday 25th Jan 2019 – Poster Sessions

19:00-19:45 **Poster Session 8** Clinical Science – **Incretin-based therapies: GLP-1 receptor agonists and DPP-4 inhibitors**

Chairman: **Daniel Quast, Bochum, Germany**

No.	Presenter	Title
PO 63	Melchiorsen, J.U. (University of Copenhagen, Copenhagen, Denmark)	Genetic variations of the GLP-1 receptor; basic pharmacological characterization to prepare for future personalized medicine for metabolic diseases
PO 64	Penshovska Nikolova, V. (Centar for Diabetes, Skopje, Macedonia)	Safety effect of combination therapy with Liraglutide plus metformin on HGP and HbA_{1c} vs each therapy alone in patients with T2DM
PO 65	Livadas, S. (Endocrine Unit, Metropolitan Hospital, Athens, Greece)	Liraglutide administration improves hormonal/metabolic profile and reproductive features in women with HAIR-AN syndrome
PO 66	Parker, V. E. R. (MedImmune, Cambridge, UK)	Effects of MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, on amino acids, ketones, and free fatty acids
PO 67	Carr, R. (MSD A/S, Copenhagen, Denmark)	Efficacy and safety of continuing sitagliptin when initiating insulin therapy in subjects with type 2 diabetes mellitus
PO 68	Carr, R. (MSD A/S, Copenhagen, Denmark)	Safety and efficacy of sitagliptin compared with dapagliflozin in patients with T2D, mild renal impairment, and inadequate glycemic control on metformin +/- a sulfonylurea
PO 69	D'Oria, R. (Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy)	Effects of DPP-4 inhibitors, alone or in combination with pioglitazone, on palmitate-induced apoptosis and autophagy in human cardiac progenitor cells from control and diabetic subjects
PO 70	Nauck, M.A. (Diabetes Center Bochum-Hattingen, St. Josef Hospital, Ruhr-University Bochum, Bochum, Germany)	Sitagliptin and cardiovascular outcomes during and after acute myocardial infarction: observations from TECOS
PO 71	Carr, R. (MSD A/S, Copenhagen, Denmark)	Early initiation of sitagliptin during metformin up-titration in treatment of patients with T2DM

6 min per poster (3 min presentation and 3 min discussion)

20:00 *Get-Together and Dinner*

Welcome speech:
***Juris* J. Meier:**
Welcome to the Ruhr-Region and its Soccer Culture

23:00 Return to hotels

Day 3 – 26th Jan 2016

08:30 - 10:00 Clinical science - **Incretins – New roles, mechanisms, and therapeutic perspectives**

Chairman: **Christophe Magnan, Paris, France**

No.	Presenter	Title	From	To
OP 23	Veedfald, S. (University of Copenhagen, Copenhagen, Denmark)	Glucose-dependent insulinotropic polypeptide is a pancreatic polypeptide secretagogue in healthy men and patients with type 2 diabetes	8:30 AM	8:40 AM
OP 24	Woelnerhanssen, B.K. (St. Clara Research Ltd St. Claraspital Basel, Basel, Switzerland)	Dose response trial with xylitol and erythritol: CCK, PYY and GLP-1 release and gastric emptying in healthy humans	8:45 AM	8:55 AM
OP 25	Christensen, AS. (Steno Diabetes Center Copenhagen, Hellerup, Denmark)	Both GIP and GLP-1 potentiate sulfonylurea-induced insulin secretion in patients with HNF1A-diabetes	9:00 AM	9:10 AM
OP 26	Ellingsgaard, H. (University Hospital Copenhagen, Copenhagen, Denmark)	GLP-1 secretion is regulated by IL-6 signalling: a randomized, placebo-controlled study	9:15 AM	9:25 AM
OP 27	Jonsson, A. (University of Copenhagen, Copenhagen, Denmark)	Genome wide association study (GWAS) of circulating levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) during an oral glucose tolerance test	9:30 AM	9:40 AM
OP 28	Bjerre Knudsen, L. (Novo Nordisk, Copenhagen, Denmark)	Oral absorption of peptide drugs	9:45 AM	9:55 AM

10 min presentation and 5 min discussion

10:00 - 10:30 Break

Until 12:00 Check-Out Hotels

Day 3 – 26th Jan 2016 (continued)

10:30 - 12:00 Clinical science - **GLP-1, GIP and glucagon: Pathophysiology and therapy**

Chairwoman: **Helga Ellingsgaard, Copenhagen, Denmark**

No.	Presenter	Title	From	To
OP 29	Barbosa-Yañez, R.L. (DIfE, Nuthetal, Germany)	Endogenous glucagon secretion in response to carbohydrate- or protein-rich meals is not regulated by endogenous GIP or GLP-1 in healthy or type 2 diabetic humans	10:30 AM	10:40 AM
OP 30	Baekdal, M. (Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark)	Glucagon secretion from the human gastrointestinal tract	10:45 AM	10:55 AM
OP 31	Meyer-Gerspach, A.C. (St. Clara Research Ltd at St. Claraspital, Basel, Switzerland)	Role of endogenous GLP-1 in the central regulation of appetite in healthy lean individuals	11:00 AM	11:10 AM
OP 32	Torekov, SS. (University of Copenhagen, Copenhagen, Denmark)	Patients with obesity caused by melanocortin-4 receptor mutations can be treated with a glucagon-like peptide-1 receptor agonist	11:15 AM	11:25 AM
OP 33	Meier, J.J. (Diabetes Center Bochum-Hattingen, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany)	Effects of sequential treatment with lixisenatide, insulin glargine, or their combination on meal-related glycaemic excursions, insulin and glucagon secretion, and gastric emptying in patients with type 2 diabetes	11:30 AM	11:40 AM
OP 34	Bergmann, N. (Steno Diabetes Center Copenhagen, Copenhagen, Denmark)	The effects of GIP on food intake, energy expenditure, appetite and plasma glucose in patients with type 2 diabetes treated with a long-acting GLP-1 receptor agonist	11:45 AM	11:55 AM

10 min presentation and 5 min discussion

Day 3 – 26th Jan 2016 (continued)

12:00 - 13:30 Clinical science - **Incretins and their therapeutic derivatives: Beneficial and adverse effects**

Chairman: **Juris J. Meier, Bochum, Germany**

No.	Presenter	Title	From	To
OP 35	Boer, GA. (University of Copenhagen, Copenhagen, Denmark)	Effects of acute and sub-chronic inhibition of glucose-dependent insulinotropic polypeptide on lipid metabolism	12:00 PM	12:10 PM
OP 36	Lorza-Gil, E. (Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Zentrum München at the Univ. of Tübingen; German Center for Diabetes Research DZD e.V., Germany)	Reaggregation of human islet cells improve beta-cell function	12:15 PM	12:25 PM
OP 37	Grespan, E. (CNR Institute of Neuroscience, Padua, Italy)	The different mechanisms of GIP and GLP-1 action explain their different therapeutic efficacy in diabetes	12:30 PM	12:40 PM
OP 38	Nauck, M.A. (Diabetes Center Bochum-Hattingen, St. Josef-Hospital, Ruhr University, Bochum, Germany)	Effects of liraglutide versus placebo on gallbladder events: results from the LEADER trial	12:45 PM	12:55 PM
OP 39	Abd El Aziz, M. (Diabetes Center Bochum-Hattingen, St. Josef Hospital, Ruhr-University Bochum, Bochum, Germany)	Neoplasms after therapy with incretin-based glucose-lowering medications (GLP-1 receptor agonists and inhibitors of dipeptidyl peptidase-4) – a systematic safety analysis	1:00 PM	1:10 PM
OP 40	Helsted, M. M. (Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark)	The role of the incretin hormones in postprandial bone remodelling	1:15 PM	1:25 PM

10 min presentation and 5 min discussion

Day 3 – 26th Jan 2016 (continued)

13:30 - 13:45	**Closing Remarks,** **Presentation of Awards** **(for the oral presentations and posters)** Michael A. Nauck, Bochum, Germany
13:45 - 14:00	**Invitation to the Forth EASD Study Group Meeting** Chairperson 2019-2021 (Bernard Thorens, Lausanne, Switzerland)
14:00	*Lunch/lunch packages* for departing participants
14:15	Shuttle bus to Düsseldorf airport (a second bus will leave slightly later)

ABSTRACTS: ORAL PRESENTATIONS

BASIC RESEARCH - SECRETION AND ACTIONS OF GIP AND GLP-1

OP 1

GLP1 peptide as a vehicle for targeted delivery of antisense oligonucleotides to pancreatic ß-cells

C. Ämmälä[1], D. Bazdarevic[1], P. Andersson[1], D. Janzén[1], R. Jansson-Löfmark[1], E. Kay[1], L. Knerr[1], T. Prakash[2], P. Seth[2], J. Meuller[1], L. Sundström[1], C. Wennberg-Huldt[1], S. Andersson[1]

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Failure of the pancreatic islet to compensate for increasing insulin resistance by expanding functional mass is a key hallmark in the development of type 2 diabetes (T2D). Many genome wide association studies have identified genetic variants affecting β-cell function or insulin secretion. Currently, therapies restoring functional β-cell mass remain an unmet medical need; and are therefore a key objective in the development of novel, potential cures for T2D. Antisense oligonucleotides (ASO), a new emerging drug modality, hold promise as a means of silencing the expression of genes linked to reduced functional β-cell mass in T2D. However, development of novel ASO based treatment is hampered by the resistance of pancreatic islets to the uptake of ASOs following systemic administration.

We have recently shown that ASO conjugation to a GLP1 receptor (GLP1R) agonist enhances the productive uptake of ASO in pancreatic β-cells both in vitro and in vivo, resulting in potent reduction of target gene levels. The productive uptake of ASOs is dependent on GLP1R expression as the effect is lost in GLP1 receptor knockout mice. Furthermore, in vitro, GLP1-ASO conjugates are much more potent in a native β-cell environment (MIN6c4, IC50=0.3nM) compared to GLP1R overexpressing cell lines (HEK293, IC50=0.1µM). In vivo, GLP1R expressing cells in tissues other than the pancreas are also less effective in internalizing ASO cargo and reducing target gene expression.

We have demonstrated, in vitro and in vivo, that GLP1R acts as a homing beacon for efficient delivery of ASO cargo to pancreatic β-cells with enhanced productive uptake relative peripheral tissues, and that the uptake of ASO mediated by the GLP1R appears to be dependent on the cellular milieu of insulin secreting β-cells.

OP 2

Glucagon-like peptide 1 targeted delivery of estrogen redifferentiates functional β-cell mass in diabetes

S. Sachs[1], A. Bastidas-Ponce[1], S. Tritschler[1], M. Bakhti[1], A. Böttcher[1], MA. Sánchez-Garrido[1], M. Kleinert[2], K. Fischer[1], S. Jall[1], A. Harger[1], S. Brandt[1], M. Tarquis-Medina[1], E. Bader[1], S. Roscioni[1], S. Ussar[1], B. Finan[3], R. DiMarchi[3], MH. Tschöp[1], F. Theis[1], SM. Hofmann[1], TD. Müller[1], H. Lickert[1]

[1]Helmholtz Zentrum München, Munich, Germany; [2]University of Copenhagen, Copenhagen, Denmark; [3]Indiana University, Bloomington, Indiana

Insulin-dependent diabetes is characterized by β-cell loss and dysfunction. Treatments regenerating β-cell mass have high disease modifying and curative potential. Dedifferentiation of β-cells has been proposed as a functional mechanism underlying β-cell failure in type 1 and 2 diabetes. We previously reported the design and preclinical evaluation of a molecule (GLP-1/estrogen) that delivers the nuclear hormone estrogen specifically into GLP-1 receptor positive cells, thereby improving body weight and islet glucose metabolism without detrimental estrogen effects in GLP-1R negative tissues (Finan et al., Nat Med 2012).

Here, we assessed the pharmacological potential of GLP-1/estrogen to improve islet function under conditions of streptozotocin (STZ) induced hyperglycemia in mice. Our data show that GLP-1/estrogen has superior efficacy relative to the individual GLP-1 and estrogen monotherapies to improve hyperglycemia accompanied by increased fasting c-peptide and insulin levels and improved islet histology. Single cell RNA sequencing (scRNAseq), performed 110 days after STZ treatment, revealed substantial STZ-induced β-cell dedifferentiation, which was reversed by daily GLP-1/estrogen treatment but not by GLP-1 or estrogen alone. GLP-1/estrogen induced restoration of β-cell mass, function and identity was characterized by restored

expression of established mature β-cell marker such as Urocortin 3 and Glut2, while expression of immature marker decreased. Of note, the scRNAseq revealed a so far unknown panel of robust genetic marker indicative of β-cell de- and redifferentiation, which robustly increased following STZ-induced β-cell dedifferentiation and decreased with GLP-1/estrogen induced β-cell redifferentiation.

In summary, we here demonstrate for the first time that GLP-1/estrogen has high pharmacological potential to improve β-cell redifferentiation under conditions of STZ-induced hyperglycemia and describe the identification of new β-cell maturity marker.

OP 3

Agonist-induced palmitoylation of the GLP-1R regulates receptor recruitment to the lipid rafts, internalisation and signalling

S. Bitsi[1], T. Buenaventura[1], W. E. Laughlin[1], T. Burgoyne[2], Z. Lyu[1], A. I. Oqua[1], H. Norman[1], A. S. Klymchenko[3], I. R. Jr. Corrêa[4], A. Walker[1], A. Inoue[5], A. Hanyaloglu[1], G. A. Rutter[1], S. R. Bloom[1], B. Jones[1], A. Tomas[1]

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Palmitoylation is a key post-translational modification that regulates G protein-coupled receptor (GPCR) translocation to cholesterol-rich membrane microdomains, known as lipid rafts, which act as trafficking and signalling hotspots. Here, we investigate whether agonist-dependent palmitoylation and lipid raft recruitment of the glucagon-like peptide-1 receptor (GLP-1R), a key GPCR target for diabetes therapy, modulates its trafficking and signalling properties.

SNAP-GLP-1R-expressing cells were treated with the pharmacological agonist exendin-4 or with “biased” exendin-4 derivatives with altered trafficking and signalling profiles, the GLP-1R positive allosteric modulator BETP, and/or MβCD, which causes raft disruption via cholesterol depletion. GLP-1R clustering and internalisation were measured by electron and confocal microscopy and HTRF-based assays. β-arrestin recruitment was examined using a complementation-based method, and GLP-1R palmitoylation with a capture-based assay. Gαs and GLP-1R levels in detergent-soluble and -resistant membrane fractions were determined by immunoblotting. Total and raft-specific protein kinase A and cAMP signalling were measured with FRET-based biosensors.

Exendin-4 binding led to GLP-1R palmitoylation, clustering and recruitment into lipid rafts. In comparison, Ex-phe1, an agonist biased away from internalisation and β-arrestin recruitment, resulted in decelerated palmitoylation, reduced clustering and attenuated signalling from lipid rafts. Full responses were restored when Ex-phe1 was co-administered with BETP. Site-directed mutagenesis of the GLP-1R palmitoylation site or MβCD-induced raft disruption caused defects in receptor clustering and cAMP responses. While lipid raft partitioning was a determining factor for GLP-1R endocytosis, primarily via clathrin-coated pits, β-arrestins appeared to be dispensable.

These findings could be exploited for the pharmacological optimisation of GLP-1R signalling in diabetic patients.

OP 4

Potentiation of satiation by glucagon-like peptide-1 producing preproglucagon neurons

D.I. Brierley[1], F. Reimann[2], F.M. Gribble[2], S. Trapp[1]

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GLP-1-producing preproglucagon (PPG) neurons in the nucleus tractus solitarii (NTS) suppress food intake when chemogenetically activated. Conversely, when PPG neurons are inhibited or ablated intake is increased, but only under certain conditions. It remains to be determined if: A) they have a role in satiation and/or satiety under physiological conditions; and B) whether their activation suppresses intake by potentiation of satiation and/or satiety, or induction of nausea, as commonly associated with GLP-1 analogues.

To address this, transgenic Glu-Cre mice were transduced with Cre-dependent hM3Dq or hM4Di DREADD receptors in the NTS, and effects of acute PPG neuron activation (hM3Dq) or inhibition (hM4Di) on feeding were recorded using automated pellet dispensers and video coding of behavioural satiety sequences (BSS).

In free-feeding hM4Di mice, inhibition did not affect intake, however it was increased in the first hour of refeeding after an overnight fast ($p<0.01$), due to increased meal size ($p<0.05$), but not frequency. When given Ensure liquid diet for 1hr, inhibition increased intake ($p<0.05$) by increasing feeding duration ($p<0.01$) but not frequency, and subsequent 1hr chow intake was decreased ($p<0.05$).

In free-feeding hM3Dq mice, hourly intake was reduced during hours 1-5 ($p<0.05$-0.0001), and cumulative intake at hours 3-48 ($p<0.001$-0.0001), without compensatory refeeding. This effect required activation of ~80% of transduced neurons, vs <1% in controls (by cFos i.r., $p<0.0001$). In overnight fasted mice, activation reduced 40 min intake ($p<0.05$) and advanced the onset of satiety (15-20 min to ≤5 min), with the typical BSS maintained.

PPG neurons thus appear to comprise a secondary physiological satiation circuit, and have the capacity to potentiate satiation/satiety. Supraphysiological stimulation of these neurons may thus represent an effective anti-obesity strategy.

OP 5

Functional effects of biased oxyntomodulin analogues

P. J. Pickford, M. A. Lucey, J. S. Minnion, S. R. Bloom, B. J. Jones

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Introduction: Oxyntomodulin (OXM) is a naturally occurring incretin with agonist activity at both the glucagon and glucagon-like peptide-1 receptors (GCGR and GLP-1R). Due to its ability to reduce body weight and improve glucose homeostasis, interest in its analogues as a potential therapy for obesity is gathering. However, the potentially beneficial impact of “biased signalling”, wherein different intracellular signalling pathways can be preferentially activated or diminished, on OXM responses is yet to be investigated. Here we describe the physiological effects of a biased oxyntomodulin analogue *versus* an unbiased equivalent both *in vitro* and *in vivo*.

Methods: A screen of OXM analogue peptides was performed to determine bias between cyclic AMP and beta-arrestin responses in PathHunter CHO-K1 cells overexpressing either human GCGR or GLP-1R. Chronic signal duration was also measured in vitro in hepatocyte-like (Huh7) and pancreatic beta cell (INS-1 832/3) cell lines. Impact on appetite regulation and glucose tolerance was determined by acute food intake studies in lean and diet-induced-obese (DIO) C57BL/6 mice.

Results: A single amino acid substitution toward the N-terminus of the peptide consistently diminished beta-arrestin-2 recruitment at both the GCGR and GLP-1R (by 41% and 17%, respectively), with minimal effect on cAMP signalling potency. Over 16 hours in vitro, the peptides with reduced arrestin recruitment showed increased potency and efficacy at the GCGR but not the GLP-1R. In lean and obese mice, these compounds significantly increased insulin release and the ability to tolerate glucose challenges, particularly at longer exposure times. The G-protein biased molecule also resulted in greater food intake reduction in lean mice, by an average of 10% over 8 hours.

Conclusion: It may be possible to increase signalling duration of OXM analogues by shifting the signalling bias toward G-protein signalling. Consequently, consideration of biased signalling should be incorporated into intelligent drug design processes. The mechanisms linking bias to the observed physiological outcomes are currently being investigated.

OP 6

Adenosine triphosphate (ATP) is released from glucagon-like peptide 1 (GLP-1) secreting cells and signals to vagal afferent neurons

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In this study, we tested the hypothesis that GLP-1 secreting enteroendocrine cells release the small molecular neurotransmitter ATP alongside classical peptide hormones and investigated potential ATP-mediated signalling between L-cells and vagal afferent neurons.

ATP was detected in distinct punctae in GLP-1-producing L-cells and the transporter thought to be responsible for packaging ATP into vesicles, VNUT, co-localized with GLP-1 containing vesicles (20% and 25% overlap in mouse and human L-cells, respectively). In response to known GLP-1 secretogogues, ATP

release was detected using two methods: i) with a luminescence-based assay to measure ATP in culture supernatants and ii) using “sniffer patches” from HEK293 cells overexpressing $P2X_2$ channels, allowing detection of real-time ATP release. To address whether ATP released from enteroendocrine cells participates in cross-talk to neurons, we co-cultured nodose ganglion neurons with L-cells expressing Gq-DREADD, enabling cell-restricted stimulation of release in GLP-1 expressing cells by CNO. CNO triggered Ca^{2+} elevation in most L-cells as predicted, and elevated Ca^{2+} in 30% of co-cultured neurons. The broad-spectrum purinergic receptor antagonist PPADs (100μM) reduced the CNO-induced Ca^{2+} rise in nodose ganglion neurons by 68%, without abolishing responses in L-cells, supporting the idea of ATP-mediated signalling from L-cells to neurones. Finally, to examine whether L-cell-released ATP triggers afferent nerve signalling within the intact gut, we measured changes in mesenteric nerve activity from the proximal colon following angiotensin II (AngII)-mediated L-cell activation. A biphasic increase in nerve discharges was observed upon application of AngII and the plateau phase of AngII responses was significantly attenuated following pre-treatment with PPADS (83% reduction in nerve discharges, two-way ANOVA, n=4).

We have demonstrated that ATP is released from GLP-1 secreting cells and can signal to vagal afferent neurons. Our study supports a role of small molecular neurotransmitters, such as ATP, in mediating communication along the gut-brain axis.

OP 7

GIP signaling integrates with insulin signaling to regulate glucose and lipid metabolism in human adipocytes

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Recent studies suggest that activation of GIP (glucose-dependent insulinotropic peptide) receptor in conjunction with GLP-1 and/or glucagon receptors reduces adiposity and body weight in mice. Incretin actions of GIP on pancreatic beta cells are well characterized, but functions of GIP on adipose tissue are incompletely understood. Using differentiated human adipocytes we investigated GIP regulation of carbohydrate and lipid metabolism in the context of insulin signaling. GIP dose-dependently stimulated lipolysis without affecting ATGL or HSL lipolytic enzyme mRNA expression. Insulin dose-dependently suppressed lipolysis, and addition of GIP attenuated insulin effects on lipolysis without affecting Akt phosphorylation at Thr308or Ser473. Insulin administration increased lipogenesis, but GIP, in the presence or absence of insulin, had little effect on lipogenesis in [^{14}C]glucose incorporation assays. GIP increased glucose utilization in adipocytes, as measured by depletion of glucose in culture media over a 24 hour period. Notably GIP dose-dependently enhanced insulin-stimulated glucose uptake ([^{14}C]-2-deoxyglucose), but GIP alone did not appreciably regulate acute glucose uptake. We propose a new mechanistic model in which GIP signaling integrates with insulin signaling to regulate glucose and lipid metabolism. In the presence of low plasma insulin levels resembling a fasting state, GIP can increase lipolysis to mobilize nutrients from adipocytes. When insulin levels rise in a fed state, GIP can increase insulin-mediated glucose uptake in adipocytes to augment efficiency of energy storage. Further elucidation of the integration of insulin and GIP signaling will be needed to support efforts to counteract dysregulated nutrient metabolism in obesity and diabetes.

OP 8

Peptidomics to study enteroendocrine cells after bariatric surgery

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The gut is a major endocrine organ producing and secreting in response to different stimuli more than 20 different hormones. Most of these hormones are peptides that can be studied intact by mass-spectrometry. We developed a method to extract, identify and quantify these peptides from different tissues in human and mouse, and used this technique to study the effect of bariatric surgery on peptide content along the GI tract. Bariatric surgery is an important treatment for obesity due to its sustained weight loss and associated improved glucose tolerance which can partially be explained by the exacerbated post-prandial gut hormone release observed in patients after surgery. However, mechanisms to explain this specific gut hormone profile remains undetermined.

We performed vertical sleeve gastrectomy on lean mice as a model of bariatric surgery without the confounding effect of weight loss and analysed peptides from different regions of the gut, stomach and

pancreas. No change at the peptide level nor in their processing was observed. RNAseq of sorted enteroendocrine cells also showed that surgery did not alter enteroendocrine cell identity which was only dependent on their region of origin. We also reproduced these results from jejunal biopsies from a cohort of lean humans undergoing gastrectomy with Roux-en-Y reconstruction, indicating that surgery and different nutrient flows are not altering enteroendocrine cell identities. However, in mice, we could correlate the differences of intestinal transit with the strong increase of gut hormone release observed after surgery.

Our study therefore showed that bariatric surgery increases gut hormone release after food intake by increasing exposure of more distal entoendocrine cells to nutrients but does not alter gut hormone production or enteroendocrine cell identities.

BASIC RESEARCH - GLP-1, GLP-1 METABOLITES, BIHORMONAL RECEPTOR AGONISTS, AND GLUCAGON

OP 9

Blockade of the somatostatin receptor subtype 2 and 5 increases GLP-1 secretion, resulting in GLP-1 receptor-mediated lowering of blood glucose in mice

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Somatostatin (SS) inhibits the secretion of many hormones including the incretin hormone, glucagon-like peptide-1 (GLP-1) by binding to and activating one or more of the five SS receptors (SSTr). We hypothesized that antagonizing the SSTrs on enteroendocrine L-cells may increase secretion of GLP-1 and, via a GLP-1 receptor mediated mechanism, lead to lowering of blood glucose. We investigated the impact of SSTr2 and 5 on GLP-1 secretion using the perfused proximal small intestine from mice (n=6-7) by intra-arterial infusion of specific antagonists (SSTr2a and SSTr5a). We also performed oral glucose tolerance tests (OGTT) on mice (n=5-8) after administering 1mg/ml SSTr2a or SSTr5a with and without 1mg/ml of the GLP-1 receptor antagonist exendin(9-39) or 1mg/ml of a DPP-4 inhibitor (Valpyr), to investigate the role of SSTr2 and 5 on blood glucose profiles. Both antagonists dose-dependently increased GLP-1 secretion ex vivo, with incremental outputs of 114.3±27.9 fmol/15min, 421.5±95.2 fmol/15min and 502.5±68.3 fmol/15min for the SSTr2 antagonist, and 304.7 ± 53.3 fmol/15min, 963.4 ± 172.9 fmol/15min and 1973 ± 251.5 fmol/15min for the SSTr5 antagonist at 100 nM, 1 µM and 10 µM, $p<0.01$. Both SSTr2 and SSTr5 antagonists significantly reduced blood glucose during an OGTT compared to the control ($p=0.0018$ and $p=0.0022$ respectively). The addition of exendin(9-39) together with either the SSTr2a or SSTr5a completely eliminated the reduction in blood glucose ($p<0.01$). Combining SSTr2a with Valpyr significantly improved glucose tolerance compared to the PBS (AUC PBS 34.06 (mmol/l)×min, AUC SSTR2a+Valpyr 9.8 (mmol/l)×min, $p=0.0003$), whereas combining SSTr5a with Valpyr only showed a minor synergistic effect. Our results demonstrate that in the perfused intestine, SSTr5a was the strongest inducer of GLP-1 secretion. However, in vivo, SSTr2a was more potent in lowering blood glucose after an OGTT, especially in combination with Valpyr. The effect of the antagonists was completely removed when blocking GLP-1 receptor suggesting that the improved effect is indeed mediated by GLP-1.

OP 10

Liraglutide controls insulin secretion via the enteric nervous system through vagus nerve to beta cell axis

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Aim: Endogenous glucagon-like peptide-1 (GLP-1) regulates glucose-induced insulin secretion through both direct beta cell-dependent and indirect enteric neural gut-brain axis dependent pathways. Despite a lot of

data showing the endocrine mode of action of the GLP-1 receptor agonist, liraglutide, its neuronal mode of action is not well-known.

Methods: we studied the acute effects of liraglutide on insulin secretion and vagus nerve activity in healthy, vagotomized, cisplatin-treated, GLP-1 receptor knock-out high fat diet-induced and genetically-induced (db/db) diabetic mice. We also analyzed the chronic effect of liraglutide on the vagus nerve activity and the enteric nervous system structure in the mouse model of diabetes.

Results: Liraglutide dose dependently increased oral glucose-induced insulin secretion, but the incretin effect was mild when glucose was administered i.p in healthy mice. The effects of liraglutide on insulin secretion were abolished in GLP-1 receptor knock-out mice, in vagotomized and in cisplatin-treated mice indicating the involvement of the GLP-1 receptor, the vagus nerve and the enteric neurons on its mode of action. The importance of the vagus nerve is further supported by the increase in the vagus nerve firing rate following liraglutide administration, without and with concomitant administration of glucose, in a early and late phase after glucose administration. The vagus nerve activity was associated with an increase of insulin secretion in both phases. Importantly, in high fat diet-fed diabetic mice, liraglutide was able to increase insulin secretion after an oral glucose tolerance test. However, the agonist was unable to induce the first phase of vagus nerve firing rate and it was associated with a loss of the first phase of insulin secretion. After a chronic treatment, liraglutide was able to restore the first phase of vagus nerve firing rate in diabetic mice and it was associated with an higher number of enteric neurons within the gut.

Conclusions: Altogether, our findings show that liraglutide 1) requires a functional neuronal gut brain-beta cell axis to regulate glucose-induced insulin secretion in healthy and diabetic mice 2) acts as a neuroprotective molecule in chronic treatment to induce a better connection between the gut and the brain.

OP 11

A genetic screen identified hypothalamic IL-1R signaling as a regulator of insulin-induced glucagon secretion

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Glucose is the major source of energy for the brain. Thus, homeostasic processes are required to maintain blood glucose around 5 mM. Hypoglycemia detection occurs in hypothalamic glucose sensing neurons, leading to activation of autonomous nervous system and glucagon release by the alpha cells. Therefore, identification of mechanisms controling central glucose sensing is critical.

We aimed at identifying novel actors that could regulate insulin-induced glucagon secretion. We peformed an unbiased screen of this trait in a large panel of recombinant mice. Transcriptomic analysis of hypothalamus combined with quantitative trait loci (QTL) mapping showed that Irak4 was the most likely candidate. Indeed, this gene was 1) contained in the QTL on chromosme 15 that controls the trait; 2) found to be expressed in hypothalamus where glucose sensing neurons are located; and 3) its hypothalamic expression was highly correlated to the trait.

Irak4 is a kinase acting downstream of Il-1β/Il-1R signaling. Between C57BL/6J and DBA/2J mice, Il-1β and Irak4 were more expressed in hypothalamus from DBA/2J mice. This was associated with a marked decreased in insulin-induced glucagon secretion. Thus, we hypothetized that hypothalamic Il-1β/Il-1R signaling was more activated in DBA/2J mice, leading to a decreased in insulin-induced glucagon secretion.

We showed that in this strain, central inhibition of Il-1R signaling led to an increased in insulin-induced glucagon secretion. These effects were neither observed when Il-1R signaling was blocked in a systemic way nor in C57BL/6J mice. Neuronal activation analysis showed that central inhibition of Il-1R signaling increased the number of c-fos positive cells during hypoglycemia in the paraventricular nucleus of the hypothalamus (PVN). Based on our results, we can conclude that in DBA/2J mice, Il-1R signaling acts in the PVN to prevent insulin-induced neuronal activation, leading to a decreased in insulin-induced glucagon secretion.

OP 12

Elucidating the effect of the GLP-1 metabolite, GLP-1(9-36) on glucagon secretion in mouse and human islets

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Although alpha-cells express negligible proportion of GLP-1 receptor (GLP-1R), GLP-1 strongly inhibits glucagon secretion. Moreover, active GLP-1 has a very short half-life and undergoes rapid enzymatic degradation to form the metabolite, GLP-1(9-36). GLP-1(9-36) constitutes the majority of circulating GLP-1 but has no binding affinity to the classical GLP-1R. Whether GLP-1(9-36) is biologically-active has not been evaluated in depth.

Using isolated mouse and donor human islets, we have explored the regulation of glucagon by GLP-1 and its metabolite. We demonstrate that physiological concentrations of GLP-1 (10pM) can potentiate glucose-induced insulin secretion (GIIS) and inhibit glucagon release. However, GLP-1(9-36) is unable to affect GIIS but like its full-length counterpart, it potently suppresses glucagon secretion. To test the involvement of the GLP-1R, islets from GLP-1 receptor knockout mice were used to study the effects of GLP-1 and its amide on hormone release. We found that GLP-1 and its metabolite failed to affect GIIS but both amides significantly inhibited glucagon release, implying the existence of a GLP-1R-independent mechanism distinct to the alpha-cells.

We have data from a recombinant yeast system indicating that both GLP-1 and GLP-1 (9-36) are able to stimulate the GCGR. Further, the G-protein coupled receptor GPR119 which promotes GLP-1 release, is reported to be highly expressed in the alpha-cells. We have explored GPR119 and the GCGR as a potential mediator of the GLP-1R-independent effects of GLP-1 on glucagon secretion. Secretion studies with islets isolated from GPR119 knockout mice resulted in sustained inhibitory effects of GLP-1(7-36) and GLP-1(9-36), ruling out GPR119 as a mediator of GLP-1 effects. Co-application of a GCGR-specific antagonist led to the reversal of the inhibitory effects of GLP-1(9-36) on glucagon secretion. Moreover, GLP-1 and its metabolite failed to significantly block glucagon release in islets isolated from GCGR knockout mice. These observations indicate that GLP-1(9-36) may regulate glucagon secretion via the GCGR in the islet alpha-cells. Targeting this mechanism may constitute a novel therapeutic approach to address the problem of excess glucagon observed in diabetes.

OP 13

Gut microbiota dysbiosis of type 2 diabetic mice impairs the intestinal circadian rhythm of GLP-1 sensitivity

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Insulin secretion is controlled by the gut-brain-beta cell GLP-1 dependent axis. However, the mechanisms regulating this physiological mechanism are yet unknown. Since clock genes regulate metabolism as insulin secretion, we suggested that the circadian rhythm could be interfering with GLP-1 action for the control of insulin secretion. We here show that GLP-1 sensitivity is at its worse during daylight as quantified in vivo on insulin secretion. Simultaneously the GLP-1 receptor expression within the ileum as well as indexes quantifying the neuronal network during the light period were reduced when compared to the dark period. A tight correlation was observed between the expression of GLP-1 receptor within the ileum and a subset of clock genes suggesting their importance in the regulation of the gut-brain-beta cell GLP-1 dependent axis. In addition, gut microbiota dysbiosis is a key player on the control of glucose metabolism. We evaluated its importance on the regulation of clock genes and GLP-1 sensitivity. The frequencies of ileum bacteria, particularly *Ruminococcaceae* and *Lachnospiraceae*, oscillated between the light and dark periods and were maximum during the dark period. A specific pattern of clock gene expressions was associated with a subset of bacteria frequencies. On the other hand, in two models of impaired GLP-1 sensitivity i.e the diabetic and the germ-free mice the light-associated pattern of clock genes was expressed simultaneously to a decreased frequency of *Lachnospiraceae*. Thus, GLP-1 action which is regulated by the gut microbiota associates a specific pattern of intestinal clock gene expression.

OP 14

LY3298176, a novel dual GLP-1/GIP receptor agonist improves weight loss and glycemic control in preclinical models compared to selective GLP-1R agonist

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While selective GLP-1R agonists have established multiple clinical benefits, a more desirable profile for next generation incretins would include enhanced weight loss and greater glycemic control. LY3298176, was developed as a 39 amino acid, single-molecule-dual-agonist to investigate potential advantages of adding GIPR agonsim to GLP-1R agonism. The molecule is acylated with a PK profile supporting once weekly dosing. LY3298176 demonstrates a molecular bias towards the GIP receptor in membrane binding, and HEK293, cellular models that express the cognate receptors. GIPR-/-, GLP-1R-/-, and wild type ipGTT and isolated islet glucose dependent insulin secretion studies were performed demonstrating LY3298176 is a true dual agonist in vivo and ex vivo with the ability to regulate insulin secretion and glycemic control through both receptors. In DIO mice, chronic treatment demonstrated dose dependent weight loss which was significantly greater than semaglutide, an effect driven primarily by fat mass loss compared to lean mass loss. Increased weight loss compared to semaglutide was achieved by stronger and prolonged suppression of food intake and a slight but significant increase in energy expenditure that began after 7 days of treatment. In addition to improvements in weight loss, fasting glucose was significantly lower in mice treated with LY3298176 compared to semaglutide. Taken together, this data suggest LY3298176 is a true dual agonist at the GIP and GLP-1 receptors demonstrating preclinical improvements in weight loss and glycemic control compared with a selective GLP-1R agonist. These findings support further investigation of LY3298176 as a therapeutic for diabetes and potentially obesity in humans.

CLINICAL RESEARCH – GIP, GLP-1 AND GLUCAGON ACTING ALONE OR IN COMBINATION

OP 15

Glucose-dependent insulinotropic polypeptide receptor antagonism in patients with type 2 diabetes

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Background and aims: The gut-derived incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), are released and potentiate glucose-stimulated insulin secretion following meal ingestion (the incretin effect). In patients with type 2 diabetes, the insulinotropic effect of exogenous GIP has been shown to be severely reduced. In this study, we investigated the effect of endogenous GIP on postprandial appetite and hormone responses in patients with type 2 diabetes using the novel, selective, high-affinity GIP receptor antagonist; GIP(3-30)NH_2.

Methods: On two separate study days, 10 patients with type 2 diabetes received 250-minute infusions of GIP(3-30)NH_2 (1,200 pmol/kg/min) or placebo (isotonic saline) during a liquid mixed meal test followed by an ad libitum meal in a randomised, double-blinded, placebo-controlled cross-over study. We measured plasma glucose, insulin and glucagon and evaluated appetite using visual analogue scales and end-of-study *ad libitum* food consumption.

Results: Infusion with GIP(3-30)NH_2 induced a modest reduction in postprandial insulin (19 ± 15%, p=0.010) and C-peptide (14 ± 21%, p=0.021) responses (baseline-subtracted area under the curve (bsAUC)) compared to placebo. Infusion with GIP(3-30)NH_2 did not affect postprandial plasma glucose excursions (p=0.692), postprandial plasma glucagon responses (p=0.580), appetite, satiety, or *ad libitum* food consumption compared to placebo (all p > 0.05).

Conclusion: Based on GIP receptor antagonist infusions, we show that postprandial release of endogenous GIP has little effect on postprandial insulin secretion and no glucose-lowering effect in patients with type 2 diabetes, supporting that compromised GIP signalling contributes to the reduced incretin effect typical for these patients.

OP 16

Separate and combined metabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals: Two clinical studies

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Background: The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are secreted postprandially and, amongst other physiological effects, stimulate insulin secretion. The individual and combined contributions of endogenous GIP and GLP-1 to the incretin effect have not been determined.

Methods: To assess the effects of each hormone separately and combined, the GIP receptor antagonist GIP(3-30)NH_2 and the GLP-1 receptor antagonist exendin(9-39)NH_2 were infused during either four-hour 75-g oral glucose tolerance tests (OGTT) extended with an ad libitum meal or four-hour 1894 kJ-liquid mixed meal tests (MMT). Eighteen (OGTT) and 12 healthy men (MMT) received infusions of GIP(3-30)NH_2 (800 pmol/kg/min)+exendin(9-39)NH_2 (0-20 min: 1000 pmol/kg/min; 20-240 min: 450 pmol/kg/min), GIP(3-30)NH_2, exendin(9-39)NH_2, or saline on separate days in a randomized and double-blinded order.

Results: Glucose excursions were significantly higher during co-infusion than during exendin(9-39)NH_2, GIP(3-30)NH_2, and placebo infusions while the glucose excursions during the infusion of GIP(3-30)NH_2 were higher than exendin(9-39)NH_2 and placebo infusions. During the OGTT, insulin secretion (assessed by C-peptide:glucose ratio) was reduced by 8.6±16% (exendin(9-39)NH_2), 30±17% (GIP(3-30)NH_2), and 37±16% (co-infusion) compared to placebo. During the MMT, only the co-infusion significantly reduced insulin secretion (by 27±21%). The infusions with exendin(9-39)NH_2 (alone and as co-infusion) resulted in higher glucagon levels compared to placebo during both OGTT and MMT, while GIP(3-30)NH_2 resulted in lower glucagon levels during the MMT. There were no differences in ad libitum food consumption.

Conclusion

Endogenous GIP affects postprandial plasma glucose excursions more than endogenous GLP-1, but the two hormones contribute additively to control postprandial glycemia in healthy subjects.

OP 17

Impaired brain plasticity in obesity: the effects of bariatric surgery and gut hormones

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Background and aim: Obesity and diabetes are associated with reduced plasticity in the hippocampus and impairment of memory and learning. Gut hormones play a crucial role in neuroplasticity (NP) but to which extent it can mediate obesity's effects on NP and cognition is still poorly evaluated. The aims of the study were

to evaluate: i) the effect of obesity and bariatric surgery (RYGB) on NP ii) the relationship between NP and gut hormones (GLP-1, GIP and VIP) changes 6 months (6m) after RYGB iii) the relationship between NP, BDNF, Leptin and cognitive performance.

Methods: NP was assessed testing binocular rivalry before and after 2h of monocular deprivation, which provides an index of brain plasticity in the visual cortex. NP evaluation was performed on 20 healthy volunteer (NS) and 31 obese non diabetic subjects (OB) in fasting condition. A subgroup of OB subjects (n=13; BMI 45.8±4.9 kg/m2; age 43.7±9.5 years; HbA1c 41.5±5.4 mmol/mol) underwent a 75g OGTT before and 6m after RYGB. NP was performed at baseline, 1,3 and 6m after RYGB. Gut hormones, BDNF, leptin and cognitive performance were assessed at baseline and 6m after RYGB.

Results: NP was lower in OB as compared to NS (0.12±0.05 vs. 0.04±0.08, $p<0.0001$) and NP was inversely correlated with body weight ($r=-0.55$; $p<0.001$). In the OB subgroup 6 m after RYGB a significant BMI reduction (-25%; $p<0.001$) was associated with improved peripheral glucose metabolism. NP was progressively restored with a 10 fold increase of NP 6 m after RYGB ($p=0.008$). Post-OGTT GLP1 increased as well as GIP. The NP increase was correlated to active GLP-1 and negatively with GIP increase. VIP and BDNF levels did not change 6m after RYGB with no correlation with NP. Baseline BDNF was inversely correlated with fasting insulin. Post-RYGB BDNF inversely correlated with NP but positively with total and active GLP1. Fasting plasma leptin decreased (-81%; $p<0.008$) and it was inversely correlated with NP increase. NP correlated with cognitive performance. In a multiple linear regression analysis, addition of post-RYGB gut hormones, BDNF and Leptin to BMI and fasting glucose improved the r2 associated to post-RYGB NP (r2 change: 0.881; $p=0.007$).

Conclusion: obesity is associated with abnormal NP in visual cortex that can be reversed by weight-loss following bariatric surgery, supporting a strong effect of peripheral metabolism on early sensory plasticity and function. The relationship between NP increase, circulating gut hormones, BDNF and Leptin suggest a potential role of these hormones in the NP restoration and cognitive function in humans.

OP 18

Gut hormone changes post Roux-en-Y gastric bypass: One year prospective study

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Background: Bariatric surgery is currently the most effective treatment for weight loss. A key mechanism for its efficacy is the postprandial elevation of gut hormones such as GLP-1, which suppress food intake and improves insulin secretion. Literature regarding the changes in glucagon secretion is contradictory. These differences may be due to the variable performance of assays used for the measurement of glucagon levels.

Aim: To characterize the longitudinal changes in postprandial secretion of gut hormones (glucagon, GLP-1, GIP) in patients before and after Roux-en-Y gastric bypass (RYGB).

Subjects and Methods: Twenty-one obese patients with type 2 diabetes [Age: 48.2±13.2 years, BMI: 43.2±6.2 kg/m2] were studied before and at 1, 3, 6 and 12 months after RYGB. GLP-1, GIP and Glucagon levels were measured at fasting state and during a Mixed Meal Tolerance Test, using Millipore (GIP and GLP-1) and Mercodia (glucagon and GLP-1) immunoassays. The high-specificity protocol for glucagon assay (verified against a specific LC/MS-MS method) was used for this study.

Results: There was a steady increase in peak GLP-1 levels secreted in response to a mixed meal test (MMT) with time after surgery. There was no difference in glucagon secretion before and 1 month after RYGB surgery, although there was a significant decrease at 3 months and 12 months in fasting and stimulated glucagon levels after an MMT. There was no significant difference in GIP responses to MMT before and after surgery.

Conclusions: In concordance with other studies, the most consistent finding was that of an increased postprandial secretion of GLP-1 after surgery which increases in magnitude with time whereas there is no significant change in GIP secretion with surgery. At 3 and 12 months, fasted and stimulated glucagon levels fall in comparison to pre-surgical baseline, consistent with improved glucose tolerance and diabetes resolution.

OP 19

Effects of MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, on pancreatic and incretin hormones

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Background: MEDI0382, a glucagon-like peptide 1 (GLP-1)/glucagon dual-receptor agonist, is being developed for the treatment of type 2 diabetes mellitus and nonalcoholic steatohepatitis. GLP-1 receptor agonists promote glucose-dependent insulin release, suppress glucagon, and increase or have minimal effect on glucose-dependent insulinotropic polypeptide (GIP); however, knowledge of the effect on GLP-1 is limited. We characterized fasting and postprandial hormone profiles associated with MEDI0382 therapy.

Methods: In a phase 2a study (NCT02548585), 51 subjects with type 2 diabetes and a body mass index of 27–40 kg/m^2 received MEDI0382 (up-titrated to 200 µg) or placebo daily for 41 days. Glucose, insulin, glucagon, GIP, and GLP-1 were measured at baseline (day –1) and day 41 during a liquid mixed-meal tolerance test.

Results: MEDI0382 significantly reduced fasting glucose (–49.9 vs –19.2 mg/dL for placebo; $P < 0.0001$) and glucose $AUC_{0\text{-}4h}$ (–32.8 vs –10.2% for placebo; $P < 0.0001$) in association with increased fasting insulin (2.2 mU/L vs –3.9 mU/L for placebo; $P = 0.0164$), but postprandial insulin AUC was unchanged. In contrast, fasting and postprandial endogenous glucagon, GIP, and active and inactive GLP-1 were suppressed, and a delay in their kinetics was observed after MEDI0382 therapy.

Conclusion: The results suggest that MEDI0382 is insulinotropic and might also cause delayed gastric emptying. The effects of MEDI0382 on GIP have not been observed with GLP-1 receptor monoagonists and may represent a footprint of dual GLP-1/glucagon receptor agonism in this complex hormone signaling network.

OP 20

MEDI0382, a dual GLP-1 glucagon receptor agonist, promotes rapid glucose control and significant weight loss in patients with type 2 diabetes

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Background: MEDI0382 is a GLP-1/glucagon receptor dual agonist under development for the treatment of type 2 diabetes mellitus and nonalcoholic steatohepatitis. Balanced GLP-1 and glucagon receptor agonism is predicted to achieve improved glycemic control with clinically significant weight loss via increased energy expenditure and central effects on appetite.

Methods: A randomized double-blind placebo-controlled phase 2a study was undertaken to evaluate the efficacy of MEDI0382 and tolerability in different titration schedules. Subjects ($n = 65$) had type 2 diabetes mellitus and were on metformin monotherapy with an HbA1c of 6.5–8.5% and body mass index of 27–40 kg/m^2. Subjects received once-daily subcutaneous MEDI0382 up-titrated from 50 to 300 µg in one or two weekly titrations (cohorts 1 and 2, respectively) or placebo. The primary objective was to evaluate glucose AUC reduction during a mixed-meal test and body weight change in cohort 1.

Results: After 49 days of dosing, significant weight loss of 3.4% (3.3 kg) vs placebo ($P = 0.002$) was observed and 11/26 (42.3%) achieved weight loss of ≥5% ($P = 0.040$). Both postprandial and fasting glucose were significantly decreased (glucose AUC vs placebo, –27.8%, $P < 0.001$; fasting glucose vs placebo, –1.8 mmol/L, $P < 0.001$. This equated to a reduction in HbA1c of 0.6% vs placebo ($P < 0.001$). Remarkably, this improvement in glycemic parameters was evident after just 7 days of 50 µg of MEDI0382: glucose AUC, –27.4% vs placebo, $P < 0.001$; fasting glucose, –1.85 mmol/L vs placebo, $P < 0.001$. Treatment-related adverse events occurred more often with MEDI0382 (31/46 = 67.4%); most frequent was decreased appetite in 13/46 (28.3%). Nausea and vomiting were recorded in 5/26 (19.2%) and 3/26 (11.5%) after weekly titration and 7/20 (35%) and 4/20 (20%) after two-weekly titration. A significant increase in heart rate of 7.8 bpm was observed after 49 days of dosing, but there were no significant changes in systolic or diastolic blood pressure.

Conclusion: MEDI0382 administered for up to 49 days promoted significant weight loss and led to rapid reduction in both fasting and postprandial glucose levels. The tolerability profile in cohort 1 was comparable to that of marketed GLP-1 analogs; lengthening the titration interval did not improve gastrointestinal tolerability.

OP 21

LY3298176, a novel GIP and GLP-1 receptor dual agonist for the treatment of type 2 diabetes mellitus: First human dose to proof of concept in a randomised Phase 1b clinical trial

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A novel, unimolecular, long-acting dual glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptor agonist, LY3298176, has shown in preclinical models that the benefit profile obtained by selective GLP-1 receptor agonism can be improved. A Phase 1b, randomised, placebo-controlled, double-blind study was performed with LY3298176 to investigate safety and tolerability and obtain proof of concept data for clinical utility. A single-ascending dose (SAD; doses 0.25—8 mg) and 4-week multiple-ascending dose (MAD; doses 0.5—10 mg) studies were performed in healthy subjects followed by a 4-week Phase 1 b multiple-dose proof-of-concept (PoC; doses 0.5—15 mg) study in patients with T2DM. Doses greater than 5 mg were reached via titration. The most common side effects were gastrointestinal (vomiting, nausea, decreased appetite, diarrhea, abdominal distension). Side effects were observed both in healthy subjects and T2DM, dose dependent, and considered mild to moderate in severity. PK was assessed and showed dose proportionality and a half-life time of 5 days, supporting once-weekly dosing. In healthy subjects body weight was significantly reduced at doses 1.5, 4.5, and 10 mg versus placebo (LSM difference [95% CI]: -1.75 kg [-3.38, -0.12], -5.09 kg [-6.72, -3.46], and -4.61 kg [-6.21, -3.01], respectively); In subjects with T2DM fasting serum glucose was significantly reduced compared to placebo. (least square mean [LSM] difference [LY3298176 5/5/10/10 mg -49.12 mg/dL [-78.14, -20.12] and LY3298176 5/5/10/15 mg -43.15 mg/dL [-73.06, -13.21], respectively). In subjects with T2D weight was significantly reduced compared to placebo (LY3298176 5/5/10/10 mg -2.62 kg [-3.79, -1.45] and LY3298176 5/5/10/15 mg -2.07 kg [-3.25, -0.88] respectively). Based on these results, the pharmacology of LY3298176 translates from preclinical to clinical studies. LY3298176 has the potential to deliver clinically meaningful improvement in glycaemic control and body weight. The data warrant further clinical evaluation of LY3298176 for the treatment of T2DM and potentially obesity.

OP 22

Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial

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A novel dual glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptor agonist, LY3298176, was developed to assess whether the addition of GIP activity can improve the efficacy obtained with GLP-1 receptor agonism. In this double-blind, randomised, phase 2 study, patients with type 2 diabetes were assigned to receive LY3298176 (1 mg, 5 mg, 10 mg, or 15 mg), dulaglutide (1·5 mg), or placebo. The primary efficacy outcome was change in HbA1c from baseline at 26 weeks. 318 subjects were randomised. At baseline, age was 57 years, BMI 32·6 kg/m2, HbA1c 8·1%; 53% subjects were men. Changes from baseline in HbA1c with LY3298176 were -0.7% for 1 mg, -1·6% for 5 mg, -2.0% for 10 mg, and -2.4% for 15 mg, compared with 0·1% for placebo and -1.1% for dulagutide 1.5 mg. With LY3298176 up to 90% of patients treated with LY3298176 achieved the HbA1c target of less than 7·0% and up to 30 percent reached the HbA1c target of less than 5.7 percent. Changes in bodyweight ranged from -0·9 kg to -11·3 kg for LY3298176 (vs -0·4 kg for placebo, -2·7

kg for dulaglutide). With LY3298176 up to 71% of achieved weight loss of at least 5% of body weight, and up to 39% achieved weight loss of at least 10%. Gastrointestinal events were the most common treatment-emergent adverse events (23·1% for 1 mg LY3298176, 32·7% for 5 mg LY3298176, 51·0% for 10 mg LY3298176, and 66·0% for 15 mg LY3298176, 42·6% for dulaglutide, 9·8% for placebo); most events were mild to moderate in intensity and transient. LY3298176 showed clinically meaningful HbA1c reduction and weight loss of a magnitude beyond what has been demonstrated with the selective GLP-1 RA, dulaglutide. The results of this trial support a thorough evaluation of efficacy and safety in the SURPASS Phase 3 program.

CLINICAL SCIENCE
INCRETINS – NEW ROLES, MECHANISMS, AND THERAPEUTIC PERSPECTIVES

OP 23

Glucose-dependent insulinotropic polypeptide is a pancreatic polypeptide secretagogue in healthy men and patients with type 2 diabetes

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Background: Glucose-dependent insulinotropic polypeptide (GIP) is secreted postprandially from enteroendocrine cells. Studies suggest that GIP stimulates the secretion of pancreatic polypeptide (PP), an islet hormone affecting gut motility, glucose homeostasis and food intake, but in these studies the influence of ambient glucose levels on PP secretion (hyperglycaemia (hyper) is known to suppress & hypoglycaemia (hypo) to stimulate PP release) has not been taken into account.

Aim: To determine the effect of GIP on PP secretion under three glycaemic states in healthy individuals (HI) & patients with type 2 diabetes (T2D).

Methods: PP concentrations were measured in plasma from studies in which intravenous (iv) GIP (4 pmol/kg/min prime followed by 2 pmol/kg/min, 90min) & saline, were administered to HI(n=10) & T2D(n=11) during either fasting glycaemia (fasting), hyper (12-mM glucose clamp), or insulin-induced hypo. Plasma PP responses to the initial 30 min of each infusion were assessed as area under the curve (AUC).

Results: During fasting, mean plasma glucose (PG) was ~5 (HI) and 8mM (T2D). On the hypo day, target PG levels were 2.5 (HI) & 3.5mM (T2D). During fasting, PP responses were higher during iv GIP than saline in both (HI) (1.0(0.2) vs. 0.5(01)nM×min, p=0.01) and T2D (3.8(0.7) vs. 2.7(0.5)nM×min, p=0.025). During hyper, the PP response was significantly greater during iv GIP compared to saline in T2D (mean(SEM): 3.4(0.7) vs. 2.3(0.4)nM×min, p=0.001), but not in HI (0.6(0.1) vs. 0.5(0.1)nM×min, p=0.48). During declining PG levels, iv GIP increased PP levels when compared to saline in both HI (0.8(0.1) vs. 0.4(0.1)nM×min, p=0.017) & T2D (3.2(0.8) vs. 2.3(0.3)nM×min, p=0.15). When target levels of hypo were attained, PP levels surged in both groups during either infusion.

Conclusion: Infusion of GIP stimulates PP secretion in HI &T2D. Hyper blunted the stimulatory effect of GIP in HI but not in T2D – ostensibly the chosen clamp level was not permissive in HI.

OP 24

Dose response trial with xylitol and erythritol: CCK, PYY and GLP-1 release and gastric emptying in healthy humans

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Objective: With the increasing prevalence of obesity and its possible association with increasing sucrose consumption, non-nutritive sweeteners are gaining popularity. Artificial sweeteners might have adverse effects and alternative solutions are sought. Polyols such as xylitol and erythritol have been known for a long time and their beneficial effects on caries prevention and potential health benefits in diabetic patients have been

demonstrated in several studies. Incretins such as glucagon-like peptide-1 (GLP-1) and gastrointestinal peptides such as cholecystokinin (CCK) are released from the gut in response to food intake, promote satiation, reduce gastric emptying (GE) and modulate glucose homeostasis. In a previous trial with higher doses, we could demonstrate that the two sweeteners impact CCK and GLP-1 release. The aim was to study gastrointestinal peptide and incretin release as well as effects on gastric emptying in response to lower doses of xylitol and erythritol and describe a dose-response.

Methods: The study was conducted as a randomized, double-blind, parallel-group trial. A total of 12 healthy participants were included. Subjects received intragastric loads of 7g, 17g and 35g g xylitol or 10g, 25g, and 50g erythritol dissolved in 300 mL tap water; 7Solutions were enriched with 13C-sodium acetate (for determination of gastric emptying). We measured plasma GLP-1, PPY, CCK, as well as plasma insulin and glucose levels. GE was measured by a 13C-sodium acetate breath test.

Results: i) xylitol and erythritol lead to a dose-dependent increase in CCK, GLP-1 and PYY; ii) plasma insulin and glucose are not (erythritol) or minimally (xylitol) affected; iii) xylitol and erythritol induce retardation in gastric emptying rates.

Conclusion: The natural sweeteners erythritol and xylitol are low in calories, have no or only a small effect on plasma glucose and insulin release, yet stimulate gastrointestinal satiation peptides in a dose-response way. Low doses up to 50g were tolerated well without gastrointestinal discomfort.

OP 25

Both GIP and GLP-1 potentiate sulfonylurea-induced insulin secretion in patients with HNF1A-diabetes

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Background: Sulfonylurea (SU) is considered first-line treatment of hepatocyte nuclear factor 1-alpha (HNF1A)-diabetes despite risk of hypoglycaemia and potentially body weight gain. In addition, lasting glycaemic control is rarely obtained with SU why new treatments for patients with HNF1A-diabetes are needed. We evaluated the effect of exogenous glucose-dependent insulinotropic peptide (GIP) and glucagon-like-peptide 1 (GLP-1), respectively, as an add on to SU in patients with HNF1A-diabetes.

Methods: Ten patients with HNF1A-diabetes (females=4; [mean±SD] age 35.3±8.1 years; BMI 22.4±1.5 kg/m^2; HbA1c 42.3±6.6 mmol/mol) and ten matched healthy controls (females=4; age 33.9±8.0 years; BMI 22.2±2.4 kg/m^2; HbA1c 31.9±2.8 mmol/mol) were included in this randomised, placebo-controlled, cross-over study. Participants were subjected to six 2-hour glucose clamps (1 h at fasting plasma glucose (FPG) and 1 h at 1.5 × FPG). At time -90 min, participants received 1 mg of glimepiride or placebo. At time 0-120 min a continuous infusion of either GIP (1.5 pmol/l/kg), GLP-1 (0.5 pmol/l/kg) or saline (NaCl) was given. Insulin responses were quantified as baseline-subtracted area under the curves (bsAUC) from 0 to 120 min.

Results: The insulin response was attenuated in patients with HNF1A-diabetes compared to controls. In patients with HNF1A-diabetes both SU+GIP ([mean bsAUC ± SE] 7664 ± 1214) and SU+GLP-1 (6797 ± 1250) potentiated the insulin response compared to SU+NaCl (4224 ± 1280), placebo+GIP (2971 ± 531) and placebo+GLP-1 (3337 ± 803), respectively. Significant differences ($P<0.05$) in mean insulin bsAUC were observed for SU+GIP vs. placebo+GIP, placebo+GLP-1 and placebo+NaCl, respectively, and for SU+GLP-1 vs. placebo+GIP and placebo+NaCl, respectively.

Conclusion: Both GIP and GLP-1 were insulinotropic in both patients with HNF1A-diabetes and healthy subjects, and their effects were further potentiated when combined with SU.

OP 26

GLP-1 secretion is regulated by IL-6 signalling: a randomized, placebo-controlled study

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Background: Interleukin-6 (IL-6) is a cytokine with various effects on metabolism. In mice, IL-6 improved beta-cell function and glucose homeostasis via up-regulation of glucagon-like peptide 1 (GLP-1). IL-6 release from muscle during exercise potentiated this beneficial increase of GLP-1. This study aimed to identify if IL-6 has a similar effect in humans.

Methods: In a multicenter, double-blind clinical trial, we randomly assigned patients with type 2 diabetes or obesity to intravenous tocilizumab 8mg/kg every four weeks, oral sitagliptin 100mg daily, or double placebos during a 12-week training intervention. The primary endpoints were change in active GLP-1 in response to an acute exercise bout and change in the area under the concentration-time curve (AUC) of active GLP-1 during mixed meal tolerance tests at baseline and after the training intervention.

Results: A total of 19 patients were allocated to tocilizumab, 17 to sitagliptin, and 16 to placebos. During the acute exercise bout active GLP-1 levels were 26% lower with tocilizumab (multiplicative effect: 0.74 [95% confidence interval 0.56-0.98], p=0□034) and 53% higher with sitagliptin (1.53 [1.15-2.03], p=0.004) compared to placebo. After the 12-week training intervention, active GLP-1 AUC was about two-fold higher with sitagliptin compared to placebo (2.03 [1.56-2.62]; p <0.001), while GLP-1 AUC values showed a small decrease of 13% four weeks following the last tocilizumab infusion (0.87 [0.67-1.12]; p=0.261).

Conclusion/interpretation: IL-6 is implicated in the regulation of GLP-1 in humans. IL-6 receptor blockade lowered active GLP-1 levels in response to a meal and an acute exercise bout in a reversible manner without lasting effects beyond IL-6 receptor blockade.

OP 27

Genome wide association study (GWAS) of circulating levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) during an oral glucose tolerance test

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Aims/hypothesis: Large-scale genome wide association studies (GWAS) have identified multiple genetic determinants of type 2 diabetes. For many of those validated variants, it is unclear through which mechanisms they exert their effect and little is known whether these genes also affect incretin hormone levels. Our two-part aim was to examine the impact of variants associated with type 2 diabetes and glycemic traits on circulating levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) during an oral glucose tolerance test (OGTT), as well as to identify novel loci affecting those traits.

Methods: Plasma levels of GIP and GLP-1 were examined in samples obtained during an OGTT in a total of up to 2230 individuals from three different cohorts; the ADDITION-PRO cohort, the Family study and the Offspring study. Participants were given a standard 75g OGTT after an overnight fast of ≥8 hours and blood samples were drawn at 0, 30 and 120 minutes for assessment of plasma hormone levels. The incremental area under the curve (iAUC) of plasma GIP and GLP-1 were calculated from 0 to 30 minutes and from 0 to 120 minutes during the OGTT. Associations between genetic variants and plasma hormone levels were studied using a linear mixed model (EMMAX) implemented in the EPACTS software package by the use of inverse-normalized residuals of trait adjusted for age, sex and BMI in each cohort separately and were then meta-analyzed using metal.

Results: A variant in the *GIPR* locus (rs2238691) was associated with reduced circulating GIP levels after oral glucose stimulation (iAUC 0 - 30 minutes: Beta (SE) -0.255 (0.037) P= $6.5*10^{-12}$).

Conclusions/interpretation: The present meta-analysis identified a genome-wide significantly associated locus containing the GIP receptor gene (*GIPR*). This locus has previously been associated with

GIP concentration in other cohorts as well as with other diabetes related phenotypes, such as insulin secretion and BMI.

OP 28

Oral absorption of peptide drugs

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Oral administration of therapeutic peptides is hindered by poor absorption across the gastrointestinal barrier and extensive degradation by proteolytic enzymes. Here, we performed investigations focused on understanding the absorption of orally-delivered semaglutide, a glucagon-like peptide-1 (GLP-1) analog, co-formulated with the absorption-enhancer sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC) in a tablet. In contrast to intestinal absorption usually seen with small molecules, clinical and preclinical dog studies revealed that absorption of semaglutide takes place in the stomach, is confined to an area in close proximity to the tablet surface and requires co-formulation with SNAC. SNAC protects against enzymatic degradation via local buffering actions and only transiently enhances absorption. The mechanism of absorption is shown to be compound specific, transcellular and without any evidence of effect on tight junctions. These data have implications for understanding how highly efficacious and specific therapeutic peptides could be transformed from injectable to tablet-based oral therapies.

CLINICAL SCIENCE - GLP-1, GIP AND GLUCAGON: PATHOPHYSIOLOGY AND THERAPY

OP 29

Endogenous glucagon secretion in response to carbohydrate- or protein-rich meals is not regulated by endogenous GIP or GLP-1 in healthy or type 2 diabetic humans

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Exogenous application of GIP was shown to stimulate, while GLP-1 decreased glucagon secretion [1, 2]. We therefore wondered whether endogenous food stimulated release of glucagon is affected by endogenous food stimulated release of GIP or GLP-1.

In order to achieve differences in endogenous release of GIP and GLP-1, we took advantage of the very different incretin releasing properties of the glucose-fructose dimers linked by an 1,2-glycosidic bond in saccharose or a slowly cleaved 1,6-glycosidic bond in isomaltulose. While saccharose rapidly released GIP and less GLP-1, isomaltulose releases little GIP but more GLP-1 [3, 4].

We provided isocaloric breakfasts at 8:00 a.m. with 50 g saccharose or isomaltulose in water combined with either 25 g carbohydrate and 4 g protein in toast or 28 g protein in curd. The participants had normal glucose tolerance (n=10) or type 2 diabetes mellitus treated by diet or oral medication which was stopped prior to the study. A standardized evening meal was provided on the evening before the study. In response to the breakfast, the isomaltulose released significantly less GIP than the saccharose and significantly more GLP-1. Glucagon decreased after either sugar combined with toast without differences. Thus, the decrease of fasting levels of glucagon in response to carbohydrates was not affected by endogenous release of GIP or GLP-1.

The high protein breakfast with 250 g curd containing 28g milk-protein in combination with 50g saccharose further increased the GIP- but did not alter the GLP-1 responses compared to the carbohydrate breakfast. The GIP response was significantly greater after saccharose vs isomaltulose. The high protein breakfast caused a biphasic glucagon response with a smaller rapid early increase of glucagon followed by a greater and delayed

rise with maximal levels after 2h. This response was almost exactly identical after either isomaltulose or saccharose in the protein rich breakfast despite the differences of the incretins. The same experiment was performed in 10 patients with type 2 diabetes. GIP displayed significantly greater increases after saccharose as compared to isomaltulose while GLP-1 showed greater increases after isomaltulose, similar to the non-diabetic subjects. The glucagon response was monophasic, significantly greater and much more rapid in the type 2 diabetes patients compared to the controls reaching maximal levels after 30 min followed by a slow decline. Again, the differences in the incretin release did not modify the glucagon response.

We conclude that endogenously released incretins do not modify the basal or protein stimulated response of glucagon.

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OP 30

Glucagon secretion from the human gastrointestinal tract

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Background and aim: We have previously shown that totally pancreatectomised patients (PX) and patients with type 2 diabetes (T2D) exhibit hypersecretion of glucagon in response to oral glucose stimulation whereas intravenous glucose suppresses circulating glucagon concentrations. Here, we evaluated expression of the gene encoding glucagon (GCG), glucagon content and the density of glucagon-positive cells in mucosal biopsies from distinct locations in the upper gastrointestinal tract of PX patients, patients with T2D and non-diabetic controls (CTRL).

Material and methods: Eight PX patients, 8 patients with T2D and 8 sex, age and BMI-matched healthy CTRLs underwent upper enteroscopy with gastric and intestinal biopsy sampling followed by an intraluminal infusion of 100 ml 50% (v/w) glucose solution. The mucosal biopsies were subjected to full mRNA sequencing, peptide extraction and subsequent measurement of tissue glucagon content (using a mass spectrometry-validated sandwich ELISA) and immunohistochemical staining using a novel monoclonal C-terminal-specific glucagon antibody. Plasma/serum concentrations of glucose, insulin, C-peptide and proglucagon-derived hormones were measured.

Results: Small intestinal GCG expression was increased in PX and T2D (6.7 ±3.6 and 5.3±1.7 RPKM [mean±SD], p=NS) compared to CTRLs (2.4±2.1 RPKM [mean±SD], p<0.01). Furthermore, glucagon content in the small intestinal biopsies was higher in PX and T2D (31.2±12.6 and 25.8±9.8 pmol/g [mean±SD], p=NS) compared to CTRLs (2.0±1.1 pmol/g, p<0.05) and the density of glucagon-positive cells was greater in PX compared to CTRLs (31±36 vs. 2±4 cells/mm2 [mean±SD], p=0.038). Plasma glucagon responses to intraluminal glucose (as assessed by baseline-subtracted area under curve) were greater in PX and T2D (649±206 vs. 557±142 pmol/l × min [mean±SEM], p=NS) compared CTRLs (142±39 pmol/l × min, p<0.01).

Conclusion: Glucagon is found in the small intestine of PX and T2D, respectively, and likely explain their hyperglucagonaemia observed after oral glucose. The potential role of gut-derived glucagon in the pathophysiology of diabetes needs to be investigated.

Op 31

Role of endogenous GLP-1 in the central regulation of appetite in healthy lean individuals

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Introduction: Exogenous infusion of glucagon-like peptide-1 (GLP-1) or GLP-1 agonists can affect brain responses in areas involved in the regulation of appetite. In contrast, the effect of endogenous GLP-1 in the central regulation of appetite has hardly been investigated. We therefore aimed to study the effect of glucose-induced GLP-1 secretion on resting brain function in healthy individuals.

Methods: The study was conducted as randomized, cross-over trial. A total of 12 healthy participants were included. Subjects received an intragastric glucose (ig-gluc) load with or without intravenous (iv) exendin9-39 (ex9-39; specific GLP-1 receptor antagonist). Functional magnetic resonance imaging was used to investigate the effect of endogenous GLP-1 on resting state functional connectivity (rsFC) between homeostatic and reward-related brain regions. Blood samples were collected for the measurement of GI peptides, glucose and insulin. Visual analogue scales were used to rate appetite-related sensations.

Results: i) relative to ig-gluc, a significantly higher rsFC was found after iv-ex9-39/ig-gluc between the hypothalamus and the left lateral orbitofrontal cortex (OFC) as well as the left amygdala ($p \leq 0.001$, respectively); ii) relative to ig-gluc, a significantly higher rsFC was found after iv-ex9-39/ig-gluc between the right nucleus accumbens and the right lateral OFC ($p<0.001$); iii) ig-gluc significantly decreased prospective food consumption and increased fullness sensations compared to the pre-infusion baseline ($p=0.028$ and $p=0.019$, respectively), these effects were not present after iv-ex9-39/ig-gluc; iv) relative to ig-gluc, an attenuated increase in plasma insulin concentrations was found after iv-ex9-39/ig-gluc ($p=0.012$).

Conclusion: There is experimental evidence to indicate a role of endogenous GLP-1 in the central regulation of appetite. The effect is mediated by modulating rsFC in homeostatic as well as reward-related brain regions in a GLP-1 receptor-mediated fashion.

OP 32

Patients with obesity caused by melanocortin-4 receptor mutations can be treated with a glucagon-like peptide-1 receptor agonist

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Background: Pathogenic mutations in the appetite-regulating melanocortin-4 receptor (MC4R) represent the most common cause of monogenic obesity (present in 2-6 % of children with obesity) with limited treatment options. The MC4R is primarily located in the appetite-regulating center in the hypothalamus. Pathogenic mutations in the MC4R cause low MC4R functionality leading to increased appetite sensation and thereby obesity. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) cause weight loss by reducing appetite. The underlying mechanisms seem to involve GLP-1R activation in the CNS, where it enters the brain via leaks in the blood-brain-barrier (BBB) and targets GLP-1Rs in the hypothalamus. However, it is unclear which neuron populations in the hypothalamus that are involved in the action of the GLP-1RA liraglutide. We previously assessed the effect of the GLP-1 RA liraglutide 3.0 mg for 16 weeks in 14 obese individuals heterozygous for pathogenic MC4R mutations and 28 matched control participants without MC4R mutation. Liraglutide induced similar, clinically relevant weight loss of 6% in both groups, indicating that the appetite-reducing effect of liraglutide is preserved in MC4R causal obesity and that liraglutide acts independently of the MC4R pathway (Cell Metabolism July 2018, 28(1):23-32).

Aim: To confirm these observations in a morbid obese patient homozygous for pathogenic MC4R mutation.

Results: Genetic screening of >12000 patients identified a morbid obese patient homozygous for pathogenic MC4R mutation. During 16 weeks of treatment with liraglutide 3mg/daily the patient lost a total of 8.2kg (from 152.2 to 144.0). BMI was reduced from 56.5 to 53.5kg/m2. Fasting plasma glucose was reduced from 6.3 to 5.0 mmol/l. Systolic and diastolic blood pressure were reduced from 122 to 112mmHg and from 78 to 75mmHg, respectively.

Conclusion: Liraglutide acts independent of the MC4R and could be used to treat the most common form of monogenic obesity.

OP 33

Effects of sequential treatment with lixisenatide, insulin glargine, or their combination on meal-related glycaemic excursions, insulin and glucagon secretion, and gastric emptying in patients with type 2 diabetes

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Background/aims*:* It was our aim to study the effects of sequentially treating patients with type 2 diabetes with lixisenatide or insulin *glargine* alone, followed by their combination, on meal-related glycaemic excursions, insulin and glucagon secretion, and gastric emptying.

Patients/methods: 28 patients with metformin-treated type 2 diabetes were randomly assigned two treatment sequences with examinations at baseline, after treatment with either lixisenatide (10 µg once daily before breakfast for 2 weeks, followed by 20 µg/day) or insulin *glargine* (injected at bedtime, titrated to optimize fasting plasma glucose to 4.4-5.6 mmol/l) alone for 4 weeks, and after 4 weeks on a combination of both treatments. We report on 11 patients in each group who participated in all three experiments and for whom valid measurements of gastric emptying (^{13}C-octanoate breath tests) were available. After each treatment period, plasma glucose, insulin, C-peptide, insulin secretion rates (deconvolution) and glucagon as well as gastric emptying were determined after two identical mixed meals (500 kcal) served for breakfast and late lunch (8 h later).

Results: Two female and 20 male patients with type 2 diabetes (age: 59 ± 9 yrs., BMI 30.9 ± 3.6 kg/m^2, HbA_{1c} 7.8 ± 0.5 %) were studied. Lixisenatide mainly reduced postprandial glycaemic excursions, while insulin *glargine* mainly reduced fasting plasma glucose after breakfast ($p < 0.05$), and this was partially preserved after the late lunch ($p < 0.05$). After breakfast, lixisenatide (immediately following its injection) reduced insulin and glucagon secretion significantly, whereas insulin glargine only reduced glucagon slightly and did not affect insulin secretion. These effects were lost after late lunch. Gastric emptying was slowed significantly by lixisenatide treatment, but not by insulin *glargine* treatment after breakfast. After the late lunch, there was a trend towards accelerated gastric emptying in experiments with lixisenatide.

Conclusions: Lixisenatide decelerates gastric emptying after breakfast, leading to reduced glycaemic excursions and a reduction in insulin and glucagon secretion. The glycaemic reduction persists until after late lunch, despite a trend for accelerated gastric emptying. Insulin *glargine* reduces fasting plasma glucose and, less prominently, post-meal glycaemic excursions. The combination reduces glycaemia significantly more than either treatment alone, due to complimentary modes of action.

OP 34

The effects of GIP on food intake, energy expenditure, appetite and plasma glucose in patients with type 2 diabetes treated with a long-acting GLP-1 receptor agonist

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Background: Rodent data have shown that co-activation of the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) receptors potentiates the glucose-lowering and body weight-reducing effects of GLP-1. In patients with type 2 diabetes, the insulinotropic effect of GIP is impaired, however, it seems restorable to some extent when glycaemia is normalised. We evaluated the effects of GIP on food intake, appetite, resting energy expenditure and plasma glucose excursions in well-regulated patients with type 2 diabetes treated with a long-acting GLP-1 receptor agonist.

Methods: We examined patients with type 2 diabetes (n = 22, age 61±8.9 years, BMI 31.5±3.5 kg/m2, HbA1c 6.8±0.6% [51±7.0 mmol/mol]) in steady treatment with metformin and a long-acting GLP-1 receptor agonist in a cross-over design including two experimental days: One day with iv GIP infusion (8 pmol/kg/min

for 10 minutes and 6 pmol/kg/min for 260 minutes) and one day with iv saline (placebo) infusion. After 60 minutes of infusion, a mixed meal test was performed. Food intake (primary endpoint) was measured by an ad libitum meal at the end of each experimental day. Secondary endpoints included appetite ratings, resting energy expenditure and plasma glucose excursions during the mixed meal test.

Results: Food intake was similar between the GIP and placebo infusion (2.9±1.6 MJ vs. 2.7±1.2 MJ [mean±SD], p=0.17). No differences in appetite measures (hunger, satiety, fullness, prospective food consumption, nausea, thirst) or resting energy expenditure were observed between the interventions. Plasma glucose excursions (assessed by baseline subtracted area under curve) were significantly greater during GIP infusion compared to during placebo infusion (bsAUC 284±171 vs. 182±171 mmol/l×min, p=0.0033).

Conclusions: GIP infusion on top of treatment with a long-acting GLP-1 receptor agonist does not seem to affect food intake, appetite or resting energy expenditure but increased plasma glucose excursions compared to placebo.

CLINICAL SCIENCE - INCRETINS AND THEIR THERAPEUTIC DERIVATIVES: BENEFICIAL AND ADVERSE EFFECTS

OP 35

Effects of acute and sub-chronic inhibition of glucose-dependent insulinotropic polypeptide on lipid metabolism

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Even though the physiology of glucose-dependent insulinotropic polypeptide (GIP) has not been fully unraveled, there are strong indications that this peptide hormone has anabolic effects on lipid metabolism. Most in vivo studies have mainly focused on congenital models of altered GIP/GIPR signaling, which have inherent risk for compensatory adaptations. We have shown that GIP(3-30)NH_2, a naturally occurring truncated form of the native peptide, is a high-affinity antagonist of the GIP receptor. We generated a mouse-specific, long-acting variant of GIP(3-30)NH_2 aiming at creating a tool to investigate the role of GIP in lipid metabolism, thereby avoiding the adaptation of the animals to a congenital lack of GIP/GIPR.

Our preliminary results show that administration of GIP(3-30)NH_2 decreases the glucose- lowering effect of GIP during an intraperitoneal glucose test in mice ($p<0.0005$). From these positive results and results of our pharmacokinetic studies on both GIP and the antagonist, we designed a study focusing on lipid metabolism, through both acute and sub-chronic experiments.

Acute effects of the antagonist on lipid absorption following an oral lipid challenge are being investigated using [^{14}C]-oleic acid, which allows us to trace absorbed lipids in both plasma and tissues. We expect the antagonist to cause a decreased absorption of lipids into especially adipose tissue, and possibly also in tissues such as liver and muscle.

In our sub-chronic studies, animals will be treated for 8 weeks with the antagonist whilst receiving a high-fat diet. This treatment is expected to result in a reduced bodyweight compared to placebo treatment, and we expect to see a reduced fat mass in treated mice. Adipose tissue, liver, muscle and heart will be examined for tissue weight and triglyceride content, and we will visualize intracellular lipid droplets. Plasma samples will be analyzed for triglyceride levels. If our hypothesis that GIP is adipogenic holds true, we expect these values to decrease in mice treated with the GIP antagonist.

In conclusion, the availability of long-acting, highly specific GIP(3-30)NH_2 analogs enables us to investigate the possible anabolic role of GIP on lipid metabolism, both with acute and sub-chronic studies.

OP 36

Reaggregation of human islet cells improve beta-cell function

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Isolated human islets are used for transplantation with limited success. Recently, the use of reaggregated pseudoislets for transplantation resulted in an improved glucose homeostasis. This observation suggests that the reaggregation of islet cells into small clusters could be beneficial for the outcome of transplantation. In this study we compared the regulation of insulin secretion and the transcriptome of isolated human islets from organ donors and of pseudoislets generated from the same donors. Human islets obtained from ECIT centers were cultured overnight and glucose-, fatty acid- and cAMP-stimulated insulin secretion was assessed. From the same islet preparations pseudoislets were prepared and subjected to the same functional tests. In addition a comparative transcriptome analysis between islets and pseudoislets was performed.

The glucose-induced insulin secretion (GIIS) was significantly improved in pseudoislets (8-11-fold) when compared to intact islets (1.8-fold). Pseudoislets kept insulin mRNA levels and content and remained functional even after 2-weeks of culture. Stimulation of adenylyl cyclase with 5 µM forskolin further increased GIIS of pseudoislets 1.9-fold. Palmitate, 600 µM, augmented insulin secretion at 2.8 mM and 12 mM glucose 8- and 2.1-fold, respectively. Adrenaline inhibited GIIS as well as the effect of palmitate. Comparing the transcriptome of islets and pseudoislets, the islet specific transcription factors MAFA and PAX4 are upregulated. The increased SLC2A2 (GLUT-2) and ABCC8 (SUR1) mRNA levels could contribute to reduced basal and improved glucose responsiveness of pseudoislets. The mRNA levels of FFAR1 and ADRA2A were high and significantly increased in pseudoislets. The mRNA levels of other inhibitory receptors, SSTR1, SSTR3 were also higher in pseudoislets than in islets while GLP1R, SSTR2 and SSTR5 mRNA levels did not change significantly. In addition, the reaggregation was accompanied by a differential expression of extracellular matrix which may support pseudoislet formation and function. Markers of endothelial cells, acini and duct cells were reduced.

In conclusion, pseudoislets are functionally superior over isolated and cultured human islets and therefore may be a suitable tool not only for in vitro functional tests but also for transplantation.

OP 37

The different mechanisms of GIP and GLP-1 action explain their different therapeutic efficacy in diabetes

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The reduced incretin effect in type 2 diabetes (T2D) represents an important cause of postprandial hyperglycaemia, but the different pharmacologic efficacy of its major players, GLP-1 and GIP, remains unexplained. At cellular level the mechanisms activated by the two hormones and the defects of T2D are still poorly understood. We have extended a recently developed mathematical model of the β-cell to: 1) investigate the role of incretins at the cellular level on Ca^{2+} signalling and on the glucose mediated amplifying pathway (AP); 2) characterise incretin action in vivo in subjects with normal glucose tolerance (NGT) or T2D; 4) provide an explanation for the different insulinotropic activity of GIP and GLP-1 in T2D.

We used in vivo data from studies with: A) constant infusions of GIP or GLP-1 at basal glucose; B) hyperglycaemic clamps with boluses or constant infusion of GIP or GLP-1; C) a graded glucose infusion test with constant infusion of GLP-1; D) an OGTT and isoglycaemic intravenous glucose infusions with GIP or GLP-1 infusion. In the β-cell model, we hypothesize that GIP and GLP-1 increase insulin secretion rate (ISR) by a transient Ca^{2+} increase (the first 10-20 min after the incretin stimulus) and by potentiating the AP; the Ca^{2+} and glucose-dependent refilling function representing the AP is multiplied by a time-dependent factor (K_{incr}); K K_{incr} =1 without and K_{incr} 1 with incretin stimulation.

A transient Ca^{2+} increase is necessary to reproduce the transient ISR increase observed with GIP infusion at basal glucose in NGT subjects (Study A). This mechanism also accounts for the increase in early ISR during the OGTT (Study D). The amplification of the refilling function (factor K_{incr}) accounts for the sustained ISR

potentiation in all studies. The estimated transient Ca^{2+} increase was similar for GIP and GLP-1 and was preserved in T2D compared to NGT. In contrast, the effects of GIP and GLP-1 on K_{incr} had markedly different patterns: K_{incr} increased linearly with GLP-1 over a wide dose range, while with GIP K_{incr} reached a plateau already at ~80 pmol/L GIP concentration. K_{incr} sensitivity to GLP-1 was reduced by ~30% in T2D vs NGT, while for GIP the maximal K_{incr} was reduced by ~50%.

In conclusion, this study suggests that: 1) a transient rise in intracellular Ca^{2+} underlies the early effects of incretins; 2) potentiation of the AP mediates the sustained ISR; 3) in T2D the incretin effect on Ca^{2+} is preserved while the amplification of the AP is impaired; 4) saturation of GIP effects, more than impaired sensitivity, underlies the lack of insulinotropic activity of pharmacological doses of GIP in T2D.

OP 38

Effects of liraglutide versus placebo on gallbladder events: Results from the LEADER trial

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Patients with T2D have an increased gallbladder disease risk. In LEADER (NCT01179048), cholelithiasis and cholecystitis were more frequent in patients with T2D randomised to liraglutide (lira) vs placebo (PBO); this post hoc analysis explores this imbalance further.

In LEADER, 9340 patients with T2D and high cardiovascular (CV) risk were randomised 1:1 to lira 1.8 mg or PBO, plus standard of care (follow-up 3.5–5 years). Acute gallstone disease events were systematically captured and defined as gallbladder disease including biliary colic, symptomatic cholelithiasis and cholecystitis. All captured events were grouped into 4 categories: uncomplicated gallbladder stones, complicated gallbladder stones, cholecystitis (with/without gallbladder stones) or biliary obstruction. Cholecystectomy due to the events was also evaluated. Time to first event by treatment was analysed using Cox regression independently for all categories.

No clinical relevant difference were seen in patients experiencing an event in the lira vs PBO group, and in patients with vs without an event. Overall, acute gallstone disease was more common with lira vs PBO (141 patients [3.0%] vs 88 [1.9%]; HR 1.60, 95% CI 1.23–2.09), and more common with lira vs PBO for all 4 categories: uncomplicated gallbladder stone (16 [0.3%] vs 5 [0.1%]; HR 3.19, 95% CI 1.17–8.70), complicated gallbladder stone (52 [1.1%] vs 40 [0.9%]; HR 1.30, 95% CI 0.86–1.96), cholecystitis (51 [1.1%] vs 33 [0.7%]; HR 1.54, 95% CI 0.99–2.39) and biliary obstruction (25 [0.5%] vs 16 [0.3%]; HR 1.56, 95% CI 0.83–2.91). Cholecystectomy was more common in the lira vs PBO group (81 [1.74%] vs 52 [1.11%]; HR 1.56, 95% CI 1.10–2.20).

These post hoc analyses show increased incidence of gallbladder-related events with lira vs PBO treatment in 4 categories representative of different clinical diagnoses. Additionally, this correlated with increased cholecystectomy incidence.

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Neoplasms after therapy with incretin-based glucose-lowering medications (GLP-1 receptor agonists and inhibitors of dipeptidyl peptidase-4) – a systematic safety analysis

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Background/aims*:* Incretin-based glucose-lowering agents (glucagon-like peptide-1 receptor agonists; GLP-1 RAs and inhibitors of dipeptidyl peptidase-4, DPP-4 I) have been incriminated to raise the incidence of pancreatic and thyroid neoplasms. Since they have been demonstrated to stimulate ß-cell proliferation, this has been interpreted as indicating a growth-promoting role when stimulating GLP-1 receptors. It was the aim of the present analysis to scrutinize this hypothesis based clinical trial results.

Patients/methods: Manufacturers of all incretin-based drugs were asked to provide safety data with respect to neoplasms occurring in phase 2 and phase 3 clinical trials with respect to malignant neoplasms in general, and specific histological types (pancreatic and thyroid carcinoma, colorectal adenomas and carcinomas, breast cancer, prostate cancer) occurring in patients treated with GLP-1 RA or DPP-4 I versus

those treated with placebo or non-incretin comparator medications. Analogous data were retrieved from reports on cardiovascular outcomes trials with the same drugs (all compared to placebo). Rate ratios were calculated for each individual drug as well as for the classes of GLP-1 RAs and DPP-4 Is (Software: Comprehensive Metaanalysis).

Results: 47697 patients contributed 73627 patient years of observation (PYO) for GLP-1 receptor agonists (exenatide b.i.d., lixisenatide, liraglutide, exenatide q.w., dulaglutide, albiglutide, and semaglutide), and 30991 patients contributed 61143.8 PYO for placebo/comparator-treatment. 68226 patients contributed 89872 PYO for DPP-4 I (sitagliptin, vildagliptin, saxagliptin, alogliptin, and linagliptin), and 56834 patients contributed 84142 PYO for placebo/comparator-treatment. Malignant neoplasms occurred with GLP-1 RAs at a rate ratio of 0.89 (95 % confidence interval, CI: 0.81-0.97), and with DPP-4 I at a rate ratio of 0.91 (CI 0.84-0.99). For none of the specific compounds, a significantly elevated rate ratio was found. The same was the case when specifically looking at histological types of neoplasms, particularly pancreatic and thyroid cancer.

Conclusions: Based on a large sample of patients treated with incretin-based medications for up to several years, there does not seem to be a signal indicating an increased risk for malignant neoplasms with either GLP-1 RAs or DPP-4 I in general, but also for the individual compounds. The present analysis does not support previous findings indicating a higher risk for pancreatic and thyroid cancer with incretin-based medications.

OP 40

The role of the incretin hormones in postprandial bone remodelling

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Introduction: Bone resorption is reduced following a meal and an entero-osseous axis has been suggested to be responsible for this. The individual contributions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) on bone remodelling are unknown. We used a novel high-affinity selective GIP receptor antagonist, GIP(3-30)NH_2, and the selective GLP-1 receptor antagonist exendin(9-39)NH_2 to examine the separate and combined contributions of endogenous GIP and GLP-1 to postprandial bone remodelling.

Methods: We completed two randomised and double-blinded crossover studies. In study 1, healthy men (n=18) underwent four oral glucose tolerance tests (OGTT), and in study 2, healthy men (n=12) underwent four liquid mixed meal tests (MMT). In both studies, subjects received infusions of: A) GIP(3-30)NH_2 (800 pmol/kg/min) + exendin(9-39)NH_2 (20 min of 1000 pmol/kg/min, then 450 pmol/kg/min), B) GIP(3-30)NH_2 + saline, C) exendin(9-39)NH^2 + saline; D) saline + saline. The antagonist concentrations were chosen based on respective inhibitory potencies in vitro. We measured serum levels of the bone resorption marker carboxy-terminal collagen crosslinks (CTX) and the bone formation marker procollagen type 1 amino-terminal propeptide (P1NP).

Results: During infusion with GIP(3-30)NH_2, mean CTX nadirs (% of baseline) were lower than during saline infusion (44±2.4% (D) vs 53±3.4% (B) (p<0.001) during OGTT, and 33±3.3% (D) vs 39±2.3% (B) (p=0.02) during MMT). The inhibitory effects of GIP (effect=(1-$nadir_{GIP(3-30)NH2}$/$nadir_{saline}$,)×100%) accounted for 22±4.5% and 22±7.2% of postprandial bone resorption during OGTT and MMT, respectively. There were no differences in CTX nadirs (% of baseline) during infusion with exendin(9-39)NH_2 compared to saline infusions (44±3.0% (C) vs 43±2.4% (D) (p=0.97) during OGTT, and 31±2.6% (C) vs. 33±3.3% (D) (p=0.25) during MMT). In both studies, the interventions did not affect P1NP.

Conclusion: Our results suggest that endogenous GIP is responsible for 22 % of the postprandial suppression of bone resorption in healthy men, while endogenous GLP-1 does not seem to affect postprandial bone homeostasis.

ABSTRACTS: POSTERS

POSTER SESSION 1:
BASIC SCIENCE
GLP-1 AND GIP SECRETION AND BIOLOGICAL EFFECTS

PO 1

Gut hormone plasma levels after voluntary fat intake in a mouse self-administration model

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Background and aim: Triacylglycerol (TAG) what we call common fat, is the most abundant dietary lipid. TAG is digested and absorbed by the gut in the form of fatty acids and 2-monoacylglycerol. These metabolites are also sensed by G protein-coupled receptors (GPCRs) on the enteroendocrine cells of the gastrointestinal tract. Sensing of dietary lipids in the gut is known to trigger the release of signalling peptides such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1). In contrast, dietary fat ingestion is known to decrease ghrelin plasma levels. In this project, we are looking into the gut hormone plasma levels after intragastric voluntary intake of fat.

Material and methods: C57BL/6J male mice were surgically implanted with intra-gastric catheters and were trained to self-infuse Intralipid 30 % (IL30, a soybean oil-based emulsion) through the catheter in daily one-hour sessions. When a stable baseline (five consecutive days of less than 20% variation in the number of IL30 infusions) was achieved the IL30 was substituted with Intralipid 5%, the mice that responded by increasing their number of infusions were considered trained. Plasma was collected from mice that were allowed to self-administer IL30, from mice right before the training session with IL30, and naive mice. GLP-1, CCK, GIP, and ghrelin were measured.

Results: Voluntary self-infusion of IL30 significantly increased the plasma levels of GLP-1, CCK, and GIP. Plasma levels of CCK, GLP-1, and GIP were significantly correlated while CCK and GLP-1 were positively correlated with the total number of IL30 self-infusions. A positive correlation between body weight and number of IL30 self-infusions was also observed. In contrast, a significantly negative correlation between body weight and ghrelin plasma levels could be seen.

Conclusion: CCK, GLP-1, and GIP plasma concentration levels were augmented after fat voluntary self-infusion. The correlation between the peptide plasma levels supports the hypothesis that they were co-secreted. Interestingly, both total number of self-infusions and body weight were inversely correlated with pre-session ghrelin levels indicating that ghrelin is a poor predictor for the number of fat self-infusions.

PO 2

Amino acid sensing in K and L cells from mouse duodenal organoids

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Aims: In the proximal small intestine, enteroendocrine L cells secrete glucagon-like peptide 1 (GLP-1) and K cells secrete glucose dependent insulinotropic peptide (GIP) in response to nutrients. Although GIP and GLP-1 expression has been shown to overlap it remains unclear if these hormones can be recruited differently in response to secretory stimuli. This study aims to compare amino acid sensing in K and L cells using intestinal organoid cultures.

Methods: Duodenal organoids were grown in 3D cultures from crypts isolated from the proximal small intestine of transgenic mice expressing either a cAMP sensor under the control of the proglucagon promoter (GLU-Epac2camps) or the Ca2+ sensor GCaMP3 in GIP-expressing cells (GIP-cre/ROSA26-GCaMP3). Secretion assays and single cell imaging were performed in 2D monolayer cultures.

Results: Aromatic amino acids (AAAs, 10 mM)) phenylalanine (Phe) or tryptophan (Trp) stimulated GIP secretion by 1.7-fold, and GLP-1 secretion by 2.6-fold (Phe) and 2.8-fold (Trp). Glutamine (Gln, 10 mM)

stimulated GLP-1 secretion but failed to significantly stimulate GIP secretion, whilst alanine (10 mM) did not significantly elicit either GIP or GLP-1 secretion. In the presence of forskolin and IBMX to elevate intracellular cAMP levels, the same amino acids all further stimulated GIP secretion, but only Gln further enhanced GLP-1 secretion. In organoid cultures from GLU-Epac2camps mice loaded with the Ca2+ indicator Fura2, Phe or Trp simultaneously increased Ca2+ and cAMP levels in L cells. Only a subpopulation of K cells from GIP-cre/ROSA26-GCaMP3 mice elicited Ca2+ responses to Trp (11/32 cells) or Phe (7/38 cells).

Conclusions: The molecular mechanisms underlying amino acid triggered GIP and GLP-1 secretion appear to differ. Investigations are in progress to identify the receptors and transporters involved in amino acid sensing in K and L cells.

PO 3

Mechanisms of bile acid-induced glucagon-like-peptide 1 and peptide-YY secretion from the colon

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A large number of GLP-1 and PYY producing L-cells are located in the colon, but it is unknown when and to what extent these cells secrete their products into the circulation. Since bile acids (BAs) spill over from the ileum to the colon, we decided to investigate the ability of BAs to stimulate colonic GLP-1 and PYY secretion. Using isolated perfused rat/mouse colon as well as colonic stimulation in vivo, we show that BAs potently enhance secretion of GLP-1 and PYY from the colon with average increases of 3.5 and 2.9-fold, respectively. Furthermore, we find that responses depend on BA absorption and basolateral activation of the BA-receptor, TGR5. Surprisingly, the apical sodium-dependent-bile-acid-transporter (IBAT), which serves to absorb conjugated BAs, was not required for colonic conjugated BA absorption or conjugated BA-induced peptide secretion. In conclusion, we suggest that BAs may represent a major physiological stimulus for colonic L-cell secretion.

PO 4

Dietary fiber is essential to maintain intestinal weight and L cell secretion

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Background and aim: Nutrient sensing in the gut lumen is believed to exert a direct control on the secretion of gastrointestinal peptides such as glucagon-like peptide-1 and glucagon-like peptide-2. These hormones are co-secreted from enteroendocrine L cells, principally located in the ileum and colon, and yield a synergistic intestinotropic effect. Altered nutrient content is thus a manipulatable method of studying gut hormone secretion and the resultant architectural changes in the intestine. In this study, we aim to characterize gut hormone secretion and intestinal weight in response to the removal of fiber from the diet.

Methods: 16 female C57BL/6JRj mice either received a standard chow diet or were switched to a crude fiber-deficient diet for 21 days. All animals were weighed daily and the intestinal parameters explored; including intestinal weight, morphometry and gut hormone analysis. The luminal content was collected from the intestine and analyzed for bile acid and short chain fatty acid changes by mass spectrometry, results of which will be ready in time for the conference.

Results: The removal of crude fiber from the diet resulted in a significant decrease (-22%, $p<0.01$) in relative small intestinal weight corresponding with a significant decrease in intestinal crypt depth in all anatomical areas of the small intestine ($p<0.01$). The relative colon weight was significantly decreased (-34%, $p<0.01$), but colonic crypt depth was not significantly different. Total GLP-1, measured from intestinal extracts, significantly decreased in the ileum and colon of crude fiber deficient mice (-30% and -35%, $p<0.05$ and <0.01).

Active GLP-2 was not significantly different. Total PYY was significantly decreased (-44%, $p<0.01$) in the colon of crude fiber-deficient mice.

Conclusion and perspectives: Our study shows that the presence of fiber in the diet was essential to maintain both the small intestinal and colon weight during the 21 days. The removal of fiber also led to a decreased L cell secretion profile, resulting in a significant decrease of GLP-1 and PYY in the colon. Understanding these responses may thus provide a new treatment strategy for the use of dietary fiber.

PO 5

Acute injection of the GLP-1 analog Exendin-4 in the olfactory bulb improves glucose tolerance in diet-induced obese mice.

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The incretin hormone Glucagon-like peptide-1 (GLP-1) plays a crucial role in the regulation of food intake and glucose homeostasis. In the brain, GLP-1 receptor (GLP-1R) is expressed in a wide variety of areas such as the nucleus of the solitary tract, the hypothalamus and the olfactory bulb (OB). Recent *in vitro* data showed that the principal cells of the OB (mitral cells) are excited by both GLP-1 and Exendin-4 application, reinforcing the idea that the OB is sensitive to hormonal signals regulating energy homeostasis. Thus, our study focuses on the *in vivo* effect of GLP-1R drugs on glucose homeostasis through its action in the OB.

We performed acute bilateral injections of Exendin-4 (0.5µg/µL, 1µL) or Exendin-9 (12.5µg/µL, 1µL) in the OB of lean and diet-induced obese (DIO) C57Bl6 mice. Then, we challenged mice with oral glucose (1g/kg BW) to measure their tolerance and insulin secretion.

We report that injection of Exendin-4 in the OB dramatically improves glucose tolerance of DIO mice and strongly increases their glucose-induced insulin secretion and C-peptide plasmatic concentration. Conversely, injection of Exendin-9 worsens glucose intolerance in obese mice with no change in plasmatic insulin level. Our results unravel the GLP-1 pathway in the OB as a new actor in the regulation of glucose homeostasis.

PO 6

The role of glucose-dependent insulinotropic polypeptide receptor-expressing neurons in the hypothalamus

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Background: A complex relationship exists between glucose-dependent insulinotropic polypeptide (GIP) and obesity. Implicating GIP in promoting weight gain are studies showing that blockade of GIP receptor (GIPR) protects against diet-induced obesity. Contrastingly, a novel dual GIP/GLP-1 agonist reduces food intake in rodents and body weight in rodents and man. We sought to understand how central GIP signalling affects feeding behaviour and to identify sensory pathways regulating GIP-responsive hypothalamic cells.

Method: We generated a transgenic mouse (GIPR-Cre) in which Cre expression is driven by the *Gipr* promoter, enabling the specific introduction of fluorescent labels and genetically encoded calcium sensors upon crossing with Cre reporter lines. Single-cell RNA sequencing (scRNAseq) of purified hypothalamic *Gipr* cells was used in combination with real time calcium imaging in primary cultures to probe the functional importance of key genes expressed in *Gipr* neurons. The effects of acute modulation of hypothalamic *Gipr* cell signalling on food intake was assessed after selective AAV mediated expression of Cre-dependent designer receptors exclusively activated by designer drugs (DREADDs).

Results: Staining for a fluorescent reporter expressed under the control of the *Gipr* promoter revealed that GIPR is present in the arcuate and dorsomedial nuclei of the hypothalamus. scRNAseq analysis identified 7 sub-populations of hypothalamic *Gipr* cells, including clusters exhibiting transcriptomic signatures for oligodendrocytes and neurons. The neuronal sub-population expressed receptors for gut peptides, including *Cckbr* and *Ghsr*, as well as *Lepr* and *Htr2c*. CCKBR and GHSR agonism elicited calcium responses in a subset of *Gipr* cells. Activation of G_q DREADDS in hypothalamic *Gipr* cells suppressed food intake *in vivo*.

Conclusion: *Gipr* is expressed in key feeding centres of the hypothalamus. These cells are anorexigenic, and sense peripheral signals implicated in regulating appetite.

PO 7

A GIP/GLP-1 hybrid peptide exhibits beneficial actions on hippocampal function in dietary-induced diabetic mice

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Diabetes and obesity are associated with the development of cognitive impairment and neurodegeneration and a series of studies have illustrated that both GIP and GLP-1 receptor activation can improve cognitive function. Interestingly, recent data have shown that a unimolecular multiple acting hybrid peptide of GIP and GLP-1 leads to unprecedented weight reduction and markedly improved glycaemic control in patients with T2DM. In this study, we report the impact of a GIP/GLP-1 hybrid peptide on behaviour, recognition memory, hippocampal neurogenesis and oxidative stress in diabetic mice. The hybrid peptide was constructed as follows: first 15 amino acids of (DAla2)GIP (N-terminally capped with acetyl adduct at N-terminus); amino acids 16-35 of GLP-1; 11 amino acids from the C-terminal of GIP 1-42; and 10 amino acids from the C-terminal of exendin-4. Mice administered low-dose streptozotocin (STZ) on a high fat diet received saline vehicle (0.9% (w/v); ip), (DAla2)GIP, exendin-4, a combination of both peptides or hybrid peptide (each peptide at 25 nmol/kg bw; ip) twice-daily for 28 days. Open field behaviour, novel object recognition testing, hippocampal synaptogenesis, oxidative stress and neurogenesis were evaluated, as well as, key metabolic parameters. Treatment interventions did not alter ($P>0.05$) behaviour in terms of anxiety, exploration, distance covered and speed as assessed during the open field. However, mice treated with hybrid peptide displayed significantly increased ($P<0.05$-$P<0.01$) memory recognition index, indicating improved cognitive performance. Treatment interventions resulted in significant increase ($P<0.05$-$P<0.01$) in synaptophysin staining in the polymorphic layer and stratum molecular regions. Furthermore, treatment with hybrid peptide significantly increased ($P<0.001$) synaptophysin staining in the stratum pyramidale. Treatment interventions were also associated with enhanced hippocampal neurogenesis as indicated by a significant increase ($P<0.001$) in the number of doublecortin-positive cells. Moreover, there was a significant reduction ($P<0.001$) in staining for 8-oxoguanine in both the dentate gyrus and cortex, indicative of decreased oxidative stress. Importantly, treatment with hybrid peptide significantly reduced ($P<0.05$-$P<0.001$) body weight, HbA1c, circulating glucose and insulin concentrations and significantly improved ($P<0.001$) glucose tolerance and insulin sensitivity. These data demonstrate the beneficial actions of a GIP/GLP-1 hybrid peptide to improve not only metabolic control but also aspects of memory impairment associated with diabetes-obesity. This study highlights our long-held view of therapeutic utility of GIP agonism and the need for more thorough evaluation of unimolecular multiple-acting peptides in patients.

PO 8

Roux-en-Y gastric bypass has major effects on jejunal proteomics of Zucker diabetic fatty rats

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Background: Bariatric surgery is currently the only effective cure for obesity-related type 2 diabetes. The aim of the study was to investigate the peptidomic profile of the bypassed jejunum of Zucker diabetic fatty rats that had undergone Roux-en-Y gastric bypass (RYGB) or a sham operation to probe the role of gut peptide alterations in the amelioration of diabetes.

Methods: Male Zucker diabetic fatty rats underwent RYGB or a sham operation at 12 weeks of age and were pair-fed until the termination of the study, 60 days later. Jejunal biopsies of RYGB operated (n=5) or sham operated (n=7) rats were pooled within the groups, minced, extracted using acid ethanol and concentrated for HPLC using Sep-Pak C18 cartridges. Purified peptide samples were then subjected to HPLC and fractions from all peaks with significant absorbance at 214nm were collected. Molecular masses were determined using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on the peptide peaks that showed strong differences between RYGB and sham operated groups. This was followed by MASCOT database search on the acquired MS fragmentation pattern to identify amino acid sequences and ultimately regulatory peptide entities that might play a role in diabetes remission.

Results: HPLC profile revealed >80 separate peptide peaks in each group. Fourteen peaks, eluting between 25-60 min were strikingly different in RYGB and sham groups in terms of peak height. The peptides that were decreased by RYGB were tentatively identified as neurotensin, Ras-related GTP binding protein B, leptin, metalloreductase, cytochrome C oxidase, secretin, gastrin releasing peptide (GRP), apolipoprotein C

and galectin-1. In contrast, tissue content of a number of other peptides was increased in RYGB jejunum including peptide YY (PYY), neuromodulin, potassium-transporting ATPase subunit beta, gastric inhibitory polypeptide (GIP) and beta-defensin 15.

Conclusions: These preliminary studies reveal striking differences between the peptidomic profiles in the jejunum of Roux-en-Y and sham operated Zucker diabetic fatty rats. Further investigations are required to evaluate the significance of these data and the role gut derived peptides in the amelioration of obesity-diabetes.

PO 9

Beneficial effects of GLP-1 receptor activation on metabolic control and beta-to-alpha cell transdifferentiation in multiple low-dose streptozotocin diabetes

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Islet function in diabetes is characterised by progressive loss of beta cells together with hyperglucagonaemia and alpha cell dysregulation. The present study has examined the effects of GLP-1 receptor activation with Liraglutide on islet morphology, beta-to-alpha cell transdifferentiation and metabolic control in mice with multiple low-dose streptozotocin-induced diabetes.

Methods: Diabetes was induced in 12 weeks old C57BL/6 Ins1cre/+Rosa26-eYFP mice using multiple-low-dose streptozotocin (STZ, 50mg/kg/day, 5 days). From days -2 to +10, mice (n=8) received saline or liraglutide injections (25nmol/kg, twice daily). Beta-to-alpha cell transdifferentiation was assessed by cell lineage tracing.

Results: STZ increased non-fasting glucose (26.3 ± 1.4 vs 7.7 ± 0.2mM; mean ± SEM, $p<0.001$) and reduced plasma insulin (5.7 ± 0.5 vs 9.5 ± 1.7ng/ml; $p<0.01$). This was associated with reduction in percentage islet beta-cells (58.9 ± 1.8 vs 79.4 ± 0.9%; $p<0.001$). Reciprocal increases ($p<0.001$) of alpha-cell mass (2635 ± 271.8 vs 1589 ± 156.2µm) and percentage alpha-cells (43.1 ± 2.1 vs 20.4 ± 0.9%) were observed. Increased populations ($p<0.001$) of islets with insulin negative, GFP positive cells (82.5 ± 2.5 vs 34.3 ± 4.3%) and glucagon positive, GFP positive cells (67.5 ± 2.8 vs 23.3 ± 2.9%) were detected, indicative of beta-to-alpha cell transdifferentiation. Liraglutide protected against STZ-induced hyperglycaemia (14.8 ± 1.8mM; $p<0.0001$), hypoinsulinaemia (10.5± 1.6ng/ml; $p<0.01$) and preserved islet area (7279 ± 625.8µm; $p<0.001$) with percentage beta-cells unchanged (59.3 ± 2.1%). Alpha-cell mass (3095 ± 268.8 µm) and percentage alpha-cells (44.2±2.4%) were also unchanged compared with STZ alone but fewer islets containing insulin negative, GFP positive cells (65.4 ± 4.5%; $p<0.05$) and glucagon positive, GFP positive cells (50.4 ± 5.4%, $p<0.05$) were observed.

Conclusion: Beta-cell demise and transdifferentation to alpha-cells occurs in multiple-low-dose streptozotocin diabetes and can be inhibited/reversed by GLP-1 receptor activation with Liraglutide.

POSTER SESSION 2:
BASIC SCIENCE
IDENTIFICATION OF ENTERO-ENDOCRINE CELLS AND THEIR MODE OF SECRETION

PO 10

Modelling human enteroendocrine cells using adult intestinal organoids

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Gastrointestinal (GI) enteroendocrine cells (EECs) make up the largest endocrine system in the body. The two incretin hormones Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic-polypeptide (GIP) are secreted by K and L cells, respectively and research is focussed on the physiology of these cells due to their potential value in obesity and diabetes therapy.

GI organoids are a long term model generated from adult GI-stem cells which have the potential to give rise to all epithelial lineages. Advances in technology have defined culturing conditions for both murine and human derived organoids and we have generated human organoids from different regions along the GI tract,

maintaining them for longer than 6 months. Differentiation of these lines showed EECs within 5-14 days that kept characteristics of the region of origin. As such, hormone profiles of differentiated organoids recapitulated in vivo EEC populations, for example pro-glucagon expression was higher in distal compared to proximal intestinal lines.

One key feature of EECs is the secretion of hormones in response to physiological stimuli. GI organoids, however, expose their basolateral surface to the culture medium, limiting somewhat access to apical surfaces. To overcome this we seeded differentiated organoids into 2D cultures and probed EEC responses to stimuli including GPBAR-A and Forskolin/IBMX. To better replicate in vivo architecture and analyse apico-basal responses we seeded broken up organoids onto porous transwells and differentiated them for 10-14 days. These cultures showed limited dextran (4kDa) leak, with jejunal and rectal lines showing <0.3% leaking of dextran to the basal compartment after apical application. Characterisation of these cultures showed EECs including K and L-cells with GIP and GLP-1 detected following stimulation with Forskolin/IBMX.

In conclusion, we have validated human organoid culture for different EECs populations and are beginning to probe their apico-basal responses to different stimuli.

PO 11

Neutral amino acid transporter: SLC6a19- linking amino acid sensing to gut hormone secretion?

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Aims: Neutral amino acids (AA) such as phenylalanine (Phe) are secretagogues for incretin hormone release. However, the molecular mechanism underlying AA sensing in the gastrointestinal tract is not clear. We sought to investigate the role of the apical intestinal neutral amino acid transporter SLC6a19.

Methods: Slc6a19 knock-out mice were compared to wild-type mice in oral amino acid challenge experiments and serum GIP and GLP-1 levels were measured using immunoassays. Electrical and hormonal responses to direct application of Phe to either the apical or basolateral surface of intestinal tissue were measured in Ussing chambers.

Results: In wild-type mice GIP secretion was increased within 5 mins of an oral Phe (0.5g/kg) gavage (ΔGIP: 53 +/- 17.5 pg/mL, n=10) and there was also a trend towards increased GLP1 secretion (ΔGLP-1: 3.4 +/- 3.2 pg/mL, n=3). Phe-stimulated GIP secretion was significantly blunted in Slc6a19 KO mice (Tukey test, $p<0.05$, n=6). Whilst Phe (20mM) alone did not induce secretion of GLP-1 when applied to Ussing chamber mounted jejunal tissue, subsequent GLP-1 responses to IBMX (0.2μM) + Forskolin (2μM) were significantly increased by Phe applied either apically or basolaterally compared to vehicle pre-application (Tukey test, $p<0.01$ and $p<0.0001$, respectively, n=3).

Conclusion: Our findings suggest a role for the neutral amino acid transporter SLC6a19 in acute GIP secretion. Whilst the in vivo GLP-1 responses failed to reach significance, the in vitro experiments suggest a modulatory role for L-cell secretion. Our data are compatible with the idea that Phe may need to be absorbed to reach the basolateral membrane to induce hormone secretion. Further work is underway to determine the contribution of the SLC6a19 transporter in K and L cells, using cell specific KO mice.

PO 12

Systematic mapping of tissue expression of glucagon-like peptide 1 receptor using an unbiased knock-in mouse model

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Immunostainings are widely used to investigate receptor localization. However, antibodies against g-protein coupled receptors often display inadequate specificity/sensitivity towards the specific receptor they were raised against, and thus, often cannot be trusted to correctly reveal receptor localization and abundance. Thus, the localization of the glucagon-like peptide 1 receptor (GLP-1R) is unclear because of highly variable immunohistochemical results although knowledge about its localization is essential, due to the many beneficial effects linked to GLP-1 based treatments.

In an effort to study GLP-1R localization in an antibody-free way, we developed a knock-in mouse model that utilizes the Cre-LoxP system to express a red fluorescent protein (tdTomato) in cells with GLP-1R promotor

activity. This model allows us to systematically identify, in an unbiased (antibody free) manner, GLP-1R localization by cryosectioning of excised tissue and fluorescence microscopy analysis.

Here we show unequivocal GLP-1R expression in the glands of the gastric mucosa with most signal towards the lumen (but none in parietal cell, contrary to earlier reports), in the Brunner glands and enteroendocrine cells of the duodenum, in nerve fibers in the myenteric plexus of the small intestine and colon, throughout the pancreas, in small blood vessels and tubule cells of the kidney, and in pulmonary alveoli. No signal was seen in cardiomyocytes or hepatocytes. Together these findings constitute a detailed mapping of GLP-1R localization, and provides insight to which tissues GLP-1 can effect directly.

PO 13

Alterations of enteroendocrine L- and K-cells populations with insulin deficiency and insulin resistance

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Background: Glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and glucose-dependent insulinotropic polypeptide (GIP) are gut-derived hormones that moderate important regulating actions on nutrient homeostasis. To understand the factors affecting populations of these enteroendocrine cells, and their involvement in diabetes, the present study has assessed the impact of insulin deficiency and resistance on intestinal GLP-1, PYY and GIP producing cells, together with expression of key differentiation factors.

Methods: Male C57BL/6 mice treated once daily for 5 days with low dose (50 mg/kg) streptozotocin (STZ), or 10 days with hydrocortisone (HC, 70 mg/kg), were studied. Intestinal tissues were excised on day 10. Basic morphology as well as GLP-1, GIP and PYY intestinal cell number together with Pax-6, Pdx-1, Ki-67 and TUNEL frequencies were assessed by immunocytochemistry.

Results: STZ treatment increased ($p<0.001$) blood glucose and reduced plasma insulin. HC did not affect glucose, but increased ($p<0.01$) circulating insulin and insulin resistance. Both models exhibited increased ($p<0.05$-$p<0.001$) intestinal cell crypt depth/mucosal thickness and decreased ($p<0.01$) villus width. Interestingly, STZ treatment decreased ($p<0.01$) intestinal GLP-1 and GIP cell numbers, but increased ($p<0.001$) PYY cell number. HC also decreased ($p<0.05$) intestinal GIP cells, but had no effect on GLP-1 or PYY cell numbers. Furthermore, STZ reduced ($p<0.05$) proliferation of intestinal GLP-1 and GIP expressing cells, but similar to HC, increased ($P<0.05$) proliferation of PYY cells. TUNEL positive intestinal GLP-1 cells were increased ($p<0.05$) with STZ whereas HC reduced ($p<0.01$) GIP expressing cell apoptotic frequency. Pdx-1 co-expression with GLP-1 and GIP was decreased ($P<0.05$) by STZ which also reduced ($p<0.01$) Pax-6 co-expression with GLP-1. HC had no impact on Pdx-1 and Pax-6 expression in K- and L-cells.

Conclusions: These data highlight major adaptions of the enteroendocrine system, with decreased GLP-1 and GIP cell numbers in insulin deficiency but not insulin resistance. Understanding the factors responsible for these changes could shed light on the mechanisms contributing to metabolic dysregulation in diabetes.

PO 14

Peptone mediated glucagon-like peptide 1 secretion depends on intestinal absorption and activation of basolaterally located Calcium-Sensing Receptor

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Protein is a potent stimulus for the secretion of the incretin hormone, glucagon-like peptide-1 (GLP-1), but has received much less attention than other nutrients (fat and in particular glucose) that stimulate GLP-1 secretion. The molecular mechanisms underlying protein-stimulated GLP-1 secretion are therefore not well characterized and for instance, it remains unknown whether protein stimulates secretion by activation of luminal or basolateral sensors. We studied the molecular sensing mechanisms underlying protein stimulated GLP-1 secretion using a physiologically relevant model - the isolated perfused proximal rat small intestine. This model maintains correct cell polarity and vascular integrity, thus allowing physiologically relevant studies of intestinal absorption and secretion.

Intra-luminal protein hydrolysates derived from meat (peptone; 50mg/ml) doubled GLP-1 secretion (from a basal secretion of 110±28 fmol/min). The sensory mechanisms underlying the response depended on di/tripeptide uptake through Peptide Transporter 1 (PepT1) and subsequent basolateral activation of the amino acid sensing receptor, Calcium-Sensing Receptor (CaSR), since inhibition of PepT1 as well as CaSR both

attenuated the peptone-induced GLP-1 response. Supporting this, intra-luminal peptones were absorbed efficiently by the perfused intestine (resulting in increased amino acid concentrations in the venous effluent) and infusion of amino acids robustly stimulated GLP-1 secretion. Inhibitors of voltage-gated L-type calcium-channels had no effect on secretion suggesting that peptone-mediated GLP-1 secretion is not mediated by L-cell depolarization with subsequent opening of these channels.

Our data indicate that proteins stimulate GLP-1 secretion through absorbed amino acids, which activate amino acid sensing receptors, including CaSR, situated on the basolateral membrane. Specific targeting of CaSR could serve as a target to stimulate the endogenous secretion of GLP-1.

PO 15

The contribution of TRP channels to L-cell signalling

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G protein coupled receptors (GPCRs) are essential for enteroendocrine cell sensing of a range of nutrient and neurohormonal signals. We previously demonstrated that transient receptor potential (TRP) cation channels play a role in signalling downstream of enteroendocrine GPCRs. In ileal organoid-derived L-cells, the TRPC3 inhibitor Pyr3 reduced electrical activity, intracellular Ca^{2+} elevation and GLP-1 secretion induced by free fatty acid receptor FFA1 activation (Goldspink et al, 2017).

To extend this work, we investigated the contribution of TRPC3 channels to enteroendocrine hormone secretion in response to other stimuli. GLP-1 secretion assays and single cell Ca^{2+} imaging were performed in murine intestinal primary or organoid cultures, in the presence of pharmacological TRP channel inhibitors.

In the small intestine, Pyr3 reduced GLP-1 secretion induced by postprandial lipid micelles (29.5% reduction, $p<0.0001$), peptone (41.5% reduction, $p=0.0017$), L-glutamine (44.8% reduction, $p=0.0006$) and L-tryptophan (48.4% reduction, $p<0.0001$). Non-specific TRP channel inhibitors –2-APB, flufenamic acid, SKF-96365, lanthanum and gadolinium – also attenuated GLP-1 secretion.

By contrast, initial studies suggest Pyr3 does not reduce GLP-1 secretion (n=12 wells) and Ca^{2+} signalling (n=9 cells) in response to angiotensin II, a potent colonic L-cell secretagogue. RNA sequencing analyses and quantitative RT-PCR in FAC-sorted proglucagon-expressing colonic L-cells demonstrated low *Trpc3* expression but higher levels of *Trpc1* and *Trpc4*. The contribution of these TRP channels to GPCR signalling in colonic L-cells is currently under investigation.

Our findings demonstrate the importance of TRPC3 channels in GLP-1 release from duodenal and ileal L-cells in response to fat and protein digestion products. We propose that a TRP-mediated depolarising current may be sufficient to open voltage-gated Ca^{2+} channels, evoking hormone secretion. Further work is required to assess the role of TRP channels in other enteroendocrine cell types and determine the molecular mechanisms underlying this effect.

PO 16

Tracking the development and release of hormone-containing vesicles in murine L-cells

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Gastrointestinal hormones, such as glucagon-like peptide-1 (GLP-1), are packaged within vesicles, before being transported to the cell membrane and primed for exocytosis, awaiting extra-cellular stimulation. Recently it has been suggested that different hormones are packaged into different vesicles within enteroendocrine cells. By contrast we showed that in colonic murine primary cultures and tissue sections GLP-1 is packaged predominantly within the same vesicles as Insulin-like peptide-5 (Insl5) and PeptideYY (PYY) and that these hormones are co-released to a range of stimuli. We also showed that ATP is co-released with GLP-1, but the vesicular overlap of VNUT, a transporter for ATP, and GLP-1 was limited. As, in other cells, vesicles have been shown to release their contents with multiple timescales, we aim to characterise vesicular dynamics by both total internal reflection fluorescence (TIRF) and confocal microscopy after labelling vesicles either with small fluorescent dyes such as quinacrine or by expression of fluorescent-protein-fused neuropeptideY. We developed a MATLAB-based software package (TraVIs) to allow semi-automated vesicle detection and tracking of position and intensity in time for up to three wavelengths. Currently we investigate if nutrient exposure changes vesicle dynamics and will present preliminary data.

PO 17

Classification of colonic enteroendocrine cells based on single cell mRNA expression analysis

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Colonic enteroendocrine cells (EECs) co-express glucagon-like peptide-1 (GLP-1), peptideYY (PYY) and insulin-like peptide-5 (Insl5), which we have recently demonstrated to be co-stored and co-released from a mostly overlapping vesicular pool (Billing et al. 2018). To address if different colonic enteroendocrine hormones could nonetheless be recruited differentially, we aimed to further subclassify different EECs. We used NeuroD1-CrexRosa26EYFP mice to FACsort colonic EECs for single cell mRNA sequencing. Based on their expression profile cells could be clustered into 9 groups, one of which unexpectedly had goblet cell (n=162 cells) marker expression. The remaining clusters represented somatostatin expressing D-cells (n=167), GLP-1 expressing L-cells (3 clusters) and tryptophan hydroxylase 1 expressing EC-cells (4 clusters). EC clusters differed by Secretin (n=180), Tachikinin1 (n=131) or Piezo2 (n=236 and 238) expression. Two of the L-cell clusters expressed high levels of Insulin-like peptide-5 (Insl5; n=164 and 221), whereas the remaining cluster was distinguished by co-expression of neurotensin (Nts, n=280). Immunohistochemical and LC-MS based analysis of the colon indicate that Nts expression is restricted to the proximal 3 cm of the mouse colon, whereas Insl5 expression increased towards the distal colon. Consistent with an observed relative selective expression of Agtr1a in the Insl5 L-cell and D-cell cluster, angiotensin II (10 nM) stimulated Insl5 and peptideYY release in primary epithelial cultures, but failed to stimulate Nts release, while stimulation of the more broadly expressed FFA1 with AM-1638 (3 microM) stimulated the release of all three colonic L-cell products. This demonstrates that selective recruitment of colonic hormones is feasible and other selectively expressed receptors like the Insl5-cluster enriched Olfr78 are to be investigated in the future.

POSTER SESSION 3:
BASIC SCIENCE
GUT-DERIVED PEPTIDES AND GLUCAGON

PO 18

Proglucagon peptide analysis – will the real peptide please stand up!

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The bioanalysis of proglucagon derived peptides is traditionally performed using immunoassay techniques but these are prone to cross-reactivity issues due to antibodies binding to similar sequences in other peptides. The recent application of LC-MS technologies has enabled the direct measurement of peptide hormones. The selectivity of high resolution and high mass accuracy mass spectrometers, such as the Orbitrap, can distinguish peptides with a single Dalton difference. Advances in the sensitivity of mass spectrometers (such as the triple quadrupole) have also enabled measurement of peptides such as glucagon and GLP-1(7-36 amide) in plasma. In this study we used LC-MS systems to identify which proglucagon-derived peptides are produced in the intestine and pancreas from healthy, lean mice and humans, as well as after elective gastrectomy surgery with Roux-en-Y reconstruction.

In the small intestine of humans and mice, we identified glicentin and oxyntomodulin as the major proglucagon products but were unable to detect pancreatic-type glucagon either before or after gastrectomy surgery. The only region of the gut found to produce glucagon was the stomach. By contrast, human and mouse pancreas contained large amounts of glucagon, but only low levels of glucagon-like peptides. In pancreas homogenates, GLP-1(1-37) and GLP-1(1-36 amide) predominated over the small amount of detected GLP-1(7-36 amide) that was present. Our research suggests that if glucagon and GLP-1(7-36 amide) are seen in the gut or the pancreas respectively, they are there as minor catabolites of their longer forms - oxyntomodulin and GLP-1 1-36.

PO 19

A multiplex method for quantifying gut hormone peptides using Mass Spectrometry

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The process of studying levels of gut hormone peptides in plasma is currently widely performed using immunological based detection techniques. Whilst these approaches are highly sensitive, they sometimes suffer from cross-reactivity issues with similar peptides and peptide catabolites. LC-MS based peptide detection can easily differentiate between similar peptides and is also a highly multiplexed methodology. Nano LC-MS and Orbitrap analysis of plasma (100 µL) from OGTT experiments on control, post gastrectomy and Roux-en-Y subjects was performed and was able to detect multiple peptides from multiple pro-hormones (GIP, glucagon, PYY, Neurotensin, insulin, pancreatic polypeptide, motilin and chromogranin A). The LC-MS data were subsequently compared to existing ELISA results, and gave good correlation against Insulin (R^2 = 0.96), and GIP (R^2 = 0.92). The use of nano LC-MS gives the highest possible sensitivity for peptide analysis, however each sample takes 2 hours to be run and this is not the best approach for regular clinical use. Therefore, a higher throughput method has been developed, using normal flow rate HPLC and a triple quadrupole mass spectrometer, which can detect and quantify multiple peptides in plasma sample extracts in a significantly shorter time frame. A particular focus was applied to the motilin peptide, as immunological methods for this peptide are sparse.

PO 20

Characterisation of novel gut-derived peptides in vivo and in vitro

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Currently there are over 20 known biologically active gut-derived peptide hormones involved in the regulation of appetite, glucose tolerance and gastrointestinal motility. Here we aimed to identify additional hormonal candidates utilising liquid chromatography coupled to mass spectrometry (LC-MS) to analyse the peptidome of mouse and human enteroendocrine cells (EECs). Many identified peptides are fragments from previously identified EEC secreted proteins, but we hypothesised that they might have hitherto unidentified biological activity, analogous to the existence of several pro-glucagon derived active hormones. 16 novel peptides were selected for synthesis for *in vitro* characterisation, of which 3 lead peptides were selected for further characterisation *in vivo*. A HTRF-based cAMP assay was used to assess if any of these 16 peptides were affecting cellular cAMP levels, indicating potential activity at G_i or G_s coupled receptors in a variety of cell lines, including the β cell line, INS1 832/3, but no significant effects were detectable. LC-MS was used to quantify levels of 3 peptides in plasma following subcutaneous or intravenous injection into mice to assess the pharmacokinetic parameters, indicating very short plasma half-lives ranging between 1 and 37 minutes. A study is underway to investigate the effects of chronic infusion by osmotic pump of the lead candidates on food intake and glucose tolerance in mice, the results of which are expected before Christmas. Future work will investigate if the peptide candidates activate G_q coupled pathways by measuring Ca^{2+} responses of various cell lines to application of the peptide candidates.

PO 21

Can irisin be considered an incretin-like hormone?

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Incretins are gut hormones that potentiate glucose-stimulated insulin secretion (GSIS) after meals. Likewise, we have demonstrated that the myokine irisin is released in response to a high-fat diet and enhances GSIS (Natalicchio A. et al., Diabetes 2017). In addition, similarly to GLP-1 and its analogs (GLP-1A), irisin augments insulin biosynthesis and promotes accrual of beta-cell mass. Since both irisin and GLP-1 signal via CREB/PKA and Akt in beta-cells, we investigated (i.) if irisin and GLP-1A show additive effects on GSIS, (ii.) if

irisin action on beta-cells is impaired in the presence of lipotoxicity, as shown for GLP-1/GLP-1A, and (iii.) if irisin effects involve activation of the GLP-1 receptor (GLP-1R).

To explore the additive effect of irisin and GLP-1A, INS-1E cells were stimulated with 100 nM irisin, 10 nM exendin-4 or both, for 1 h. The effects of lipotoxicity were examined following exposure to 0.5 mM palmitate for 24 h prior to irisin/GLP-1A stimulation. To investigate if irisin uses the GLP-1R, experiments were carried out following transfection with 30 nM Glp1r siRNA for 24 h. Apoptosis was detected by cytosolic release of oligosomes, insulin gene expression was evaluated by qRT-PCR, insulin content and secretion were measured by ELISA kit, and CREB phosphorylation was visualized by immunoblotting.

No additive effects on GSIS were observed when irisin and exendin-4 were used in combination. In addition, irisin-stimulated insulin biosynthesis and content and GSIS in pancreatic beta-cells were blunted following chronic exposure to palmitate, similarly to the GLP-1A. However, in the presence of GLP-1R knockdown, irisin-induced CREB phosphorylation was preserved.

In conclusion, the effects of irisin and GLP-1A on beta-cells are similar. However, irisin acts through a distinct receptor that signals in a GLP-1R-like fashion, explaining the lack of additive effects on GSIS when irisin is combined with a GLP-1A.

PO 22

Increased L-cell secretion in Gcgr-/- mice does not potentiate tumor growth

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Background: Exogenous treatment with GLP-2 has been shown to promote intestinal adenomas and adenocarcinomas in experimental models of colonic neoplasia. However, the role of endogenous GLP-2 in tumour promotion has not been established. Mice with a deletion of the glucagon receptor (Gcgr) display augmented L-cell number, elongation of the gut and an increased level of circulating total GLP-2. We hypothesized that Gcgr -/- mice had intestinal hypertrophy and were more susceptible to colonic dysplasia in a model of inflammation-induced colonic carcinogenesis.

Method: Intestinal parameters were explored in the Gcgr-/- mice and compared to their wild type littermates; including weight, morphology and proliferation. In a subsequent experiment, Gcgr-/- mice and wild type mice were given a single injection with the carcinogen azoxymethane (7.5 mg/kg) and treated with dextran sulfate sodium (DSS) in drinking water (3%) for 6 days. Bodyweight was monitored and colitis was scored by colonoscopy 18 days after DSS treatment, all animals were sacrificed in week 6. Colonic adenomas were counted under a stereomicroscope after staining with 1% methylene blue.

Results: Gcgr-/- mice showed a significant increase in wet weight of the small intestine (+33%, $P<0.01$) and the colon (+28%, $p<0.05$). Morphology showed an increase in villus height, crypt depth and mucosal area in the small intestine. Colitis score and body weight change in azoxymethane treated animals was the same in both the Gcgr-/- and wild type mice. The number of both large and small adenomas was the same in each group, with a tendency to be lower in the Gcgr -/- group.

Conclusion: Despite increased intestinal growth in the Gcgr -/- mice, we could not find any evidence that the increased L-cell secretion potentiated tumor growth.

PO 23

The effects of Glucagon on human adipose prescursors

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Background: Obesity is often associated with increased fat mass and a dysfunction of white adipose tissue. Pharmacological treatment of obesity has not yielded significant results in term of stable weight loss. Therefore, new approach based on drugs directly targeted the adipose tissue might be of interest.

Glucagon-like peptide 1 receptor agonists (GLP-1RA), such as liraglutide, are currently used for the treatment of type 2 diabetes and have been recently proposed as anti-obesity drugs, due to their relevant effects on weight loss. Recently, preclinical data showed that GLP1RA (liraglutide and GLP-1) seem to interfere with the proliferative and differentiation ability of human adipose precursors. Another key factor involved in glucose metabolism is glucagon, which as GLP-1 is a further product of the “pro-glucagon gene”.

Aim: In the present study, we investigated the activity of glucagon in an in vitro model of human adipose-derived stem cell (ASC).

Methods: We assessed the effects of glucagon on ASC proliferation by direct cell count, cytofluorimetric analysis and thymidine incorporation assay, whereas apoptosis was evaluated by cytofluorimetric analysis of annexin-V. In vitro-induced adipogenesis was assessed by specific intracellular lipid staining and gene expression of mature adipocyte markers.

Results: Glucagon administration significantly inhibited ASC proliferation, in a dose and time-dependent manner, with a maximal effect at 3 days of culture (14.0%, 25.2% and 37.1%, $p<0.01$ for 1-10-100nM glucagon, respectively). The reduction in cell proliferation observed following glucagon stimulation was not associated with apoptosis activation. In addition, increasing doses of glucagon (1-10-100nM) inhibited in vitro-induced adipogenesis, significantly lowering the intracellular lipid accumulation (-20%, -31%, -27%, $p<0.05$ for 1-10-100nM glucagon, respectively).

Conclusion: Our findings demonstrate that glucagon has an inhibitory action on the proliferation and differentiation ability of human adipose precursors. Further studies are necessary to better elucidate glucagon's action on body weight and clarify the molecular mechanisms underlying the effects of glucagon and GLP-1RA and their possible cross-talk at cellular level.

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PO 24

The amino acid citrulline does not increase blood glucose concentrations in glucagon receptor knockout mice

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Introduction: Glucagon receptor signaling is impaired (termed glucagon resistance) in patients with type 2 diabetes and fatty liver disease resulting in hyperglycemia and hyperaminoacidemia. Hepatic amino acid metabolism is partly regulated by glucagon via an increased transcription of N-acetylglutamate synthase (NAGS), an enzyme responsible for synthesis of N-acetylglutamate, the obligate activator of the first step in the urea cycle. Deficiency of NAGS in mice and humans results in hyperaminoacidemia and is mostly lethal. NAGS deficiency can be treated with carbamylglutamate, a stable variant of N-acetylglutamate.

We hypothesized that administration of carbamylglutamate and citrulline (a substrate used in ureagenesis) would increase the rate of conversion of amino acids to urea and substrates for gluconeogenesis, and thereby correct the hyperaminoacidemia observed in glucagon receptor knockout (Gcgr-/-) mice.

Methods: Blood glucose concentrations were measured in 20 Gcgr-/- mice, 18 wild-type littermates (Gcgr+/+), and 12 C57Bl/6JRj mice (all females, 9-16 weeks of age) injected intraperitoneally with either PBS or 250 mg/kg carbamylglutamate + 500 mg/kg citrulline. Additionally, urea plasma concentrations were measured in the C57Bl/6JRj mice

Results: Administration of carbamylglutamate and citrulline significantly increased blood glucose concentrations in Gcgr+/+ mice (17.6±1.5 mM vs 12.5±0.6 mM, P=0.002) and C57Bl/6JRj mice 15.0±0.7 mM vs 10.8±0.1 mM, P=0.001), but not in Gcgr-/- mice (8.3±0.3 mM vs 8.9±0.3 mM, P=0.8). In C57Bl/6JRj mice, carbamylglutamate and citrulline caused a significant increase in urea plasma concentrations (20.7±0.9 mM vs 9.6±0.5 mM, P<0.0001).

Conclusion: The amino acid citrulline is not used as substrate for gluconeogenesis in Gcgr-/- mice. This indicates that the normal coupling between the urea cycle and gluconeogenesis requires an intact glucagon receptor signaling.

PO 25

First-in-class PET tracer for the Glucagon receptor

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Background: The glucagon receptor (GCGR) is emerging as an important target in anti-diabetic therapy, especially as part of the pharmacology of dual glucagon/glucagon-like-peptide-1(GCG/GLP-1) receptor agonists. However, currently there are no suitable target engagement biomarkers for in vivo proof of binding mechanism and specificity for GCGR. For a GCG/GLP-1 receptor dual agonist, knowledge about the proportion occupied receptors in vivo could promote the understanding of the physiological effect in terms of weight loss and glycemic control. Thus, it is imperative to develop target engagement markers of GCG drug interactions in order to enable development of dual agonists. Here we describe a first-in-class GCG Positron Emission Tomography (PET) ligand, suitable for development as a tool for in vivo analysis of GCGR occupancy in the clinic.

Methods and Materials: The GCGR selective agonist peptide S01-GCG was developed from analogues of modified glucagon. Potencies to rat, Cynomolgus monkey and human GCGR were assessed by a functional cAMP assay in GCGR transfected HEK293 cells.

Chelate conjugated peptide, S01-GCG, was radiolabeled by Gallium-68(68Ga) radionucleide. [68Ga]DO3A-S01-GCG was evaluated for selectivity (co-incubation with 10µM GCG, 10µM unlabeled DO3A-S01-GCG or 1µM GLP-1), affinity (n=5) and internalization (n=3) in GCGR transfected HEK293 cells as well as in frozen liver sections from rat, Cynomolgus monkey and man by autoradiography (n=3). In vivo biodistribution, dosimetry as well as GCGR specificity studies (co-administration of 1mg/kg DO3A-S01-GCG peptide) were evaluated in Sprague Dawley rats (n=24).

Results: S01-GCG displayed functional potency for the GCGR in the range of the natural ligand but limited potency for the GLP-1 receptor. 68Ga-radiolabelling of DO3A-S01-GCG was highly reproducible with specific activity in excess of 50 MBq/nmol with high reproducibility and with radiochemical purity >95%. [68Ga]DO3A-S01-GCG binding to transfected cells and liver sections of all studied species was mediated by the GCGR with negligible cross-reactivity to GLP-1R. In cells, GCGR binding triggered internalization of [68Ga]DO3A-S01-GCG. Affinity to the human GCGR in transfected cells was 17±8 nM. In vivo, [68Ga]DO3A-S01-GCG displayed retention exclusively in liver, spleen and kidney of the rat. The binding in liver and spleen, but not kidney, was GCGR selective as it could be competed away >80% by co-injection of unlabeled DO3A-S01-GCG. The human predicted absorbed doses (kidneys 0.54 mSv/MBq and effective dose 20 µSv/MBq) allows for repeated clinical scanning annually.

Discussion: We present evidence of a first-in-class PET tracer targeting the GCG receptor, with suitable properties for clinical development. This tool has potential to provide direct quantitative evidence of GCG receptor occupancy in humans.

PO 26

Distribution of G protein coupled relaxin/insulin-like family peptide receptor-4 (RXFP4) expressing cells in the mouse brain

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Insulin-like peptide 5 (Insl5), co-secreted with glucagon-like peptide-1 from enteroendocrine L-cells in the distal region of the gastrointestinal tract, has been demonstrated to be an orexigenic hormone, with elevated levels of Insl5 following caloric restriction and Insl5 administration increasing food intake in mice (Grosse et al. (2014) PNAS). Insl5, and its cognate receptor G protein coupled relaxin/insulin-like family peptide receptor 4 (RXFP4), is therefore thought to play a role in appetite and feeding behaviour. Expanding understanding of the distribution, physiology and pharmacology of RXFP4 is essential if this receptor is developed further as a target for anti-obesity and antidiabetic drugs. For this purpose we developed a new mouse model in which Cre-recombinase is driven by the Rxfp4 promoter – this allows localization and characterization of Rxfp4-expressing cells when Cre-reporters, such as Rosa26-GCaMP3, are crossed in. Consistent with previous reports we observe Rxfp4-positive cells in the nodose ganglion, but whilst we previously failed to detect Rxfp4-expression in the central nervous system (CNS) by qRT-PCR, we now report GCaMP3 labelled cells in the

CNS. Neuronal cell bodies were concentrated in the lateral hypothalamus (LH) with apparent innervation in the median eminence (ME). Given the importance of the hypothalamus to shape feeding behaviour, this might be a key site of RXFP4 action and future studies (in collaboration with MedImmune/Cambridge) will aim to characterise the impact of selectively modulating the activity of these neurons.

POSTER SESSION 4:
BASIC SCIENCE
NOVEL ASPECTS OF INCRETIN SECRETION AND ACTION

PO 27

RXFP4, the cognate receptor for insulin-like peptide 5, alters food preference in mice

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The gut endocrine system is a central player in the control of appetite and glucose homeostasis. Insulin-like peptide 5 (Insl5), a product of colonic and rectal enteroendocrine L-cells, is elevated by caloric restriction and dose-dependently increased food intake in wildtype mice but not in mice lacking the cognate receptor G protein coupled relaxin/insulin-like family peptide receptor-4 (RXFP4). Furthermore, RXFP4-/- mice demonstrate altered feeding behaviour, particularly pronounced on a high fat diet (HFD), and food preference (Grosse et al. (2014) PNAS). Human and murine Insl5 have been shown to reduce cAMP through a pertussis toxin sensitive Gi signalling pathway in cells heterologously expressing RXFP4. Here we present a new mouse model in which Rxfp4 drives Cre-recombinase expression (Rxfp4-Cre). Crossing these with a Gi-Designer Receptors Exclusively Activated by Designer Drugs (DREADD) Cre-reporter (Rxfp4Di) and treating them with clozapine-N-oxide (CNO, 3mg/kg ip) demonstrated increased intake of liquid Ensure, a highly palatable meal (HPM). Interestingly, we demonstrated that RXFP4Dq animals displayed the opposing effect. When the highly palatable meal was offered in combination with standard laboratory chow (STD-chow), activation of the receptor expressing cells through Dq increased intake of STD- chow and reduced intake of the HPM. The effects were, however, attenuated when animals have been exposed to a HFD for two weeks. Together the data demonstrate that the Insl5-RXFP4 axis extends beyond appetite to food reward and motivation, functions typically ascribed to the central nervous system.

PO 28

Anorectic effects of biased GLP-1 receptor agonists differ with central versus peripheral administration

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We have previously shown that single N-terminal region amino acid substitutions to the exendin-4 sequence results in “biased” GLP-1R agonism: the selective targeting of G protein signalling *versus* beta-arrestin recruitment. G protein-biased agonists, with reduced beta-arrestin responses, resulted in decreased GLP-1R internalisation, faster receptor recycling, greater insulinotropic action in pancreatic beta cells, and improved anti-hyperglycaemic effects in mice.

Here, we investigated the effects of biased GLP-1R agonists on appetite regulation. Exendin-phe1 (G protein-biased) and exendin-asp3 (beta-arrestin-biased) were administered peripherally (intraperitoneal route) or centrally (intracerebroventricular route) to C57Bl/6 mice and food intake was assessed over 8 hours. Despite the better glucoregulatory profile of exendin-phe1, both agonists exhibited a similar anorectic effect when administered peripherally, albeit with a delayed onset with exendin-phe1. In contrast, after central administration, exendin-phe1 on average induced a 45% greater reduction in food intake relative to exendin-asp3.

This discrepancy in anorectic effect between peripheral and central administration of the agonists could be due to differences in their ability to access central appetite regulatory centres. We plan to investigate this possibility by comparing the brain distribution of fluorophore-conjugated biased GLP-1R agonists injected

peripherally or centrally by 3D imaging of cleared whole brains. The potential clinical relevance is that a G-protein-biased GLP-1R agonist with reduced central nervous system entry may diminish the centrally-mediated side effect of nausea that currently dose-limits type 2 diabetes treatments, whilst exhibiting enhanced insulinotropism.

PO 29

Novel anti-obesity treatment by targeting glucagon-like peptide-1 and peptide-YY in diet-induced obese mice

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Bariatric surgery is still believed the most effective treatment for obesity. Interestingly, increased levels of different enteroendocrine L-cell hormones were found in post bariatric surgery patients. Among those hormones, peptide-YY (PYY) and glucagon-like peptide-1 (GLP-1) are the most consistently elevated. In our diet-induced obesity (DIO) study, combination of various doses of Fc-conjugated GLP-1 (Fc-GLP-1) and PYY analogues were administrated to mice for 4 weeks after 16 weeks on 60% high fat diet. Co-administration of Fc-GLP-1 and PYY significantly induced weight loss from 5.5% to 26.3 % in a dose response manner, and also improved glucose tolerance, reduced body fat content as well as reduced lipid levels in a dose response manner compared to vehicle groups. Based on these results, a new molecule, Combo0402, was developed as a dual agonist for both Y2R and GLP-1 receptors. We investigated the effect of Combo0402 on food intake and glucose tolerance in wild type mice (WT) and GLP-1 receptor knockout mice (GLP-1R KO). Combo0402 significantly reduced food intake by 67% in WT lean mice and it showed a PYY-like effect in GLP-1R KO mice. Combo0402 also significantly improved glucose tolerance like GLP-1 in the WT lean mice but not GLP-1R KO mice. To better understand the anti-obesity effect of Combo0402, we used it in a DIO study. After 16 weeks on a 60% high fat diet, we administered Combo0402 at 0.15 mg.kg-1 for the first 7 days, then increased the dose to 0.5 mg.kg-1 for the last eight days of the study. Compared to PYY or GLP-1 alone, Combo0402 had a significantly greater effect on body weight, inducing a nearly 40% decrease in body weight compared to its baseline. Synergistic reductions were also seen in fasted blood glucose and body fat content. These findings suggested a synergistic effect by targeting both GLP-1 and PYY, as an effective anti-obesity treatment.

PO 30

Variations in extracellular glucose concentration elicit intracellular Ca2+ changes in GLP-1 producing preproglucagon neurons in vitro

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Preproglucagon (PPG) neurons in the nucleus tractus solitarii (NTS) produce glucagon-like peptide-1 (GLP-1) and regulate food intake. PPG neurons are activated by postprandial signals such as gastric distension and CCK, as well as leptin. However, it is unclear whether PPG neuron activity is also modulated by brain glucose levels.

This question was addressed using transgenic mice expressing the Ca2+-sensor GCaMP3 in PPG neurons. 200µm thick acute brainstem slices were cut and maintained in artificial cerebrospinal fluid (ACSF) at 30oC. GCaMP3 fluorescence was recorded to detect changes in intracellular Ca2+ ([Ca2+]i) of PPG neurons as a marker for altered neuronal activity in response to ACSF glucose concentrations ranging from 0.1mM to 10mM.

From a baseline of 1mM glucose, hypoglycaemic challenges of 0.1mM and 0.5mM glucose increased fluorescence intensity by 44±8% and 23±5%, respectively (n=19, both $p<0.001$). Conversely, increasing ACSF glucose from 1mM to 2, 3 and 5mM resulted in concentration-dependent inhibition of PPG neurons, with fluorescence intensity decreased by 9±1%, 16±1% and 19±2% (n=11/30/33, all $p<0.001$). From these data an IC50 of 0.70±0.05mM was obtained for glucose inhibition of PPG neurons. The non-metabolizable glucose analogue 2-deoxy-glucose (2-DG, at 2mM) increased fluorescence by 59±14%, revealing that 2-DG fails to substitute for glucose, but acts as an antimetabolite and inhibits glycolysis. PPG neurons thus rely on glucose metabolism to induce changes in [Ca2+]i.

PPG neurons demonstrated clear sensitivity in the physiological range of brain glucose concentrations in support of a role in detecting meal-induced glucose fluctuations. However, given that GLP-1 reduces food

intake, it is surprising that hypo- rather than hyper-glycaemia activates PPG neurons. Further analysis in vivo is needed to solve this conundrum.

PO 31

The GLP-1 receptor agonist Semaglutide lowers body weight by direct activation of hypothalamic and hindbrain mechanisms

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Semaglutide is a glucagon-like peptide 1 (GLP-1) analogue that induces up to three times more weight loss than other GLP-1 receptor agonists (GLP-1RAs) in patients with type 2 diabetes. The weight lowering effects of GLP-1RAs are reported to be mediated through GLP-1Rs in the brain. Here we show that semaglutide treatment affects food preference, reduces food intake and causes weight loss without evoking a compensatory decrease in energy expenditure (EE). Semaglutide directly accesses the brain stem, septal nucleus, and hypothalamus through a transport-mechanism which likely involves tanycytes adjacent to circumventricular organs. Semaglutide administration induced central c-Fos activation in several brain areas including hindbrain areas directly targeted by semaglutide, but also secondary areas without direct GLP-1R interaction. A fully automated, unbiased analysis of semaglutide access, c-Fos activity, GLP-1R distribution and brain connectivity revealed that much of the activation may be controlled from neurons in the lateral parabrachial nucleus. RNASeq based gene expression analysis of microdissected brain areas from semaglutide administered rats showed upregulation of prolactin releasing hormone and TH in the area postrema. We suggest that semaglutide lowers body weight by accessing and activating neurons in the hindbrain and the hypothalamus to affect appetite regulation as well as EE.

PO 32

Differential regulation of endocytic trafficking determines spatial and temporal organization of signaling from beta cell incretin receptors

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Reduced beta cell insulin secretion is the primary metabolic defect in type 2 diabetes. Class B G protein-coupled receptors (GPCRs) for the two most important insulinotropic incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are functional in beta cells, which also express the closely related glucagon (GCG) receptor (GCGR). Little is known about how multiple receptor signalling is integrated to regulate beta cell function. As spatiotemporal control of signalling is a key mechanism to regulate receptor output, we have investigated the dynamic trafficking and signalling properties of these three beta cell GPCRs. Using surface-labelled SNAP-tagged receptors and fluorophore conjugates of GLP-1, GIP and GCG, we found that the GLP-1 receptor (GLP-1R) displayed a rapid internalisation and slow recycling profile, while the GIP receptor (GIPR) showed the opposite pattern. GCGR displayed the slowest internalisation, only apparent after hours of stimulation. Consequently, receptor degradation was detected only for the GLP-1R. Furthermore, while endogenous agonist remained associated to intracellular GLP-1R and GIPR, it became rapidly disconnected from GCGR, accumulating intracellularly while the receptor remained at the plasma membrane. Co-localisation with the recycling regulator SNX27 was rapid but not sustained for internalised GLP-1Rs, while intracellular GIPR continuously associated with SNX27. Despite increased cAMP production in the absence of β-arrestins and enhanced insulin secretion following β-arrestin-2 knockdown for all three GPCRs, reliance on β-arrestins for internalisation was much greater for GIPR than for GLP-1R. Experiments are currently under way to measure endosomal versus total cAMP generation using a newly engineered endosome-targeted FRET biosensor, as well as relative propensity to associate with β-arrestin-2 and FlAsH-BRET assessments of β-arrestin-2 conformational signatures for each of the three GPCRs.

PO 33

Disclosing incretin receptor activation using molecular dynamics simulations

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Structural studies of incretins and their receptors have for a long time been dependent on mutation studies and, when it comes to ligand design; trial and error. We have seen that very subtle changes in ligand or receptor sequence can have a dramatic effect on downstream signaling pathways, among which biased signaling, that has not yet been described on a molecular level. The relatively big size of the class B GPCRs together with the lack of high-resolution crystal structures have been great limitations in using computational techniques to study them. Nowadays, however, the publication of a number of GLP-1, Glucagon and calcitonin receptor crystal structures and advances in the field of computer science (e.g. GPU accelerated molecular dynamics), allows us to study these systems *in silico*. To explain and quantify the phenomena we observe in the lab we are combining *in vitro* assays with a set of molecular dynamics simulations. We assess the stability of GIP and GLP-1 and its ligands separately and in complex with each other. What residues are involved in ligand binding and receptor activation? Are we able to show a mechanism leading to receptor activation? And can we explain biased signaling? Answering these questions and investigating these receptors on a molecular level will pave a way towards the rational design of novel ligands and modulators.

PO 34

GIP and GLP-1 increase glucose effectiveness in normal and hi-fat fed mice regardless of changes in insulin resistance

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The main processes responsible for glucose disposal following an intravenous glucose challenge are insulin secretion, insulin sensitivity (S_I; $10^{-4}min^{-1}/(pmol/l)$) and glucose effectiveness (S_G; min^{-1}) defined as the action of hyperglycemia on glucose turnover independent on elevated insulin. This study examined the effects on S_I and S_G of GIP or GLP-1 (dose 3 nmol/kg) added to intravenous glucose load (0.35 g/kg) in IVGTT performed in normal (CT, n=62; weight=23 g) and high fat fed (HF; n=52; 37 g) female C57BL/6J after 5hr standardized fasting. S_I and S_G were assessed with minimal model analysis of IVGTT glucose and insulin data.

When no incretin was added, compared to CT, HF exhibited lower insulin peak after intravenous glucose (IPK=1.0±0.1 *vs.* 1.3±0.1 nmol/l; p=0.03); S_I was reduced (0.71±0.1 *vs.* 1.14±0.1; p=0.006); while S_G did not change (0.047±0.005; p=0.3).

When incretins were added to glucose, in both CT and HF, both GIP and GLP-1, besides increasing IPK (on average 2.5±0.3; p<0.0001), enhanced S_G (0.063±0.007 and 0.058±0.007 in CT and 0.076±0.010 and 0.072±0.008 in HF, respectively; all p<0.005 compared to the relative glucose alone tests). GIP decreased S_I in both CT and HF whereas S_I did not change after GLP 1.

We conclude that in both CT and HF, GIP and GLP-1 markedly increase insulin secretion, GIP reduces S_I, while GLP-1 has no effect on S_I. Both incretins increase S_G, by 30% in CT and even more (36%) in HF. Since it is known that incretins diminish glucose production, this study yields evidence that glucose effectiveness reflects the suppression of hepatic glucose production, regardless of both insulin action and the degree of obesity.

PO 35

Myocardial infarction is sufficient to increase GLP-1 secretion leading to improved left ventricular contractility and mitochondrial respiratory capacity

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Introduction: Myocardial infarction causes rapid impairment of left ventricular function and requires a hypercontractile response of non-infarcted tissue areas to maintain hemodynamic stability. This compensatory adaptation is mediated by humoral, inflammatory and neuronal signals. GLP-1 is an incretin hormone with

glucoregulatory and cardioprotective capacities and is secreted in response to nutritional and inflammatory stimuli. Inactivation of GLP-1 is caused by the ubiquitously present enzyme DPP-4.

Methods: Circulating concentrations of GLP-1 were assessed after myocardial infarction and functional relevance evaluated under consideration of metabolism, left ventricular contractility and mitochondrial function.

Results: Circulating GLP-1 concentrations were markedly increased in patients with acute myocardial infarction. Experimental myocardial infarction by permanent LAD ligation proved sufficient to increase GLP-1 secretion in mice. This happened in a time dependent manner, which coincided with the capacity of DPP-4 inhibition (by Linagliptin) to augment left ventricular contractility in a GLP-1 receptor dependent manner. Mechanistically, DPP-4 inhibition increased AMPK activity and stimulated mitochondrial respiratory capacity of non-infarcted tissue areas.

Conclusion: We describe a new functional relevance of inflammatory GLP-1 secretion for left ventricular contractility during myocardial infarction.

POSTER SESSION 5:
BASIC SCIENCE
INCRETINS AND LIVER, BONE, KIDNEY, AND LUNGS

PO 36

Impaired hepatic glucagon signaling as a cause for non-alcoholic fatty liver disease

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Introduction: Dysregulated glucagon secretion contributes to the increased hepatic glucose production in type 2 diabetic patients. Glucagon receptor antagonists have therefore been considered for glucose lowering therapy- however, side effects including increased amounts of fat in the liver and increased plasma concentrations of low-density lipoprotein have been reported. Suggesting that glucagon plays an important role in lipid metabolism, and its impaired signaling may contribute to hepatic steatosis, is essential for understanding development of non-alcoholic fatty liver disease (NAFLD).

Objective: To assess potential alterations in lipid metabolism in various mouse models with disrupted glucagon receptor signaling.

Methods: Using RNA sequencing of liver biopsies from five glucagon receptor knockout (Gcgr-/-) and five wild-type littermates (WT) mice (all females, 11 weeks old), we identified differential regulated genes. Separately, plasma profiling of 11 Gcgr-/- mice and 11 WT littermates (all females, 8-22 weeks old) was performed using liquid chromatography mass spectrometry based metabolomics.

Preliminary results: In the livers of Gcgr-/- mice, genes involved in fatty acid synthesis (ACOT3, Fabp5, PNPLA3), and genes involved in uptake, transport, and metabolism of fatty acids and/or fatty acyl-CoAs (Abcd2, Elovl3, Cyp4a10) were upregulated when compared to WT littermates. Furthermore, the metabolomics profile of Gcgr-/- mice showed an altered plasma profile of sphingomyelins and acylcarnitines.

Future studies: To investigate further, hepatic fat and plasma concentrations of free fatty acids and triglycerides will be measured in Gcgr-/- mice, mice lacking endogenous glucagon, and mice treated with a glucagon receptor antagonist (GRA, 25-2648). Finally, mice treated with either GRA or vehicle will be challenged with olive oil (administered via oral gavage) and plasma concentrations of triglycerides and free fatty acids will be measured.

Conclusion: Exploration of the role of glucagon in lipid metabolism may contribute to our understanding of NAFLD, and provide a basis for possible new therapeutic approaches.

PO 37

The Glucagon-like peptide 2 receptor knock-out mouse and intestinal injury

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Background and aim: Gastrointestinal mucositis is an unwanted side effect to most cancer treatments. Glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2) are co-secreted from intestinal L-cells in response to food intake. GLP-2 has been demonstrated to enhance mucosal repair following intestinal damage induced by chemotherapy. However, whether the endogenous secretion of GLP-2 is physiologically essential for mucosal repair and protection remain unresolved. We have recently generated a GLP-2 receptor knockout (GLP-2R KO) mouse using CRISPR-Cas9 technology. From this model, we want to characterize the intestines and investigate if the deficiency of GLP-2R activity increases intestinal susceptibility to injury using a model of chemotherapy-induced mucositis.

Methods: Female GLP-2R KO and wild type (WT) mice will be treated with GLP-2 (25 µg x 2 for 10 days) or PBS, and intestinal parameters will be explored after sacrifice. In a subsequent study, GLP-2R KO and WT mice will receive an intraperitoneal injection of 5-fluorouracil (400 mg/kg) or saline, inducing acute mucositis after 72 hours. Following mucositis, the intestinal epithelium undergoes continuous regeneration via proliferation, differentiation, and migration of the cells. Therefore, this particular model is excellent for the study of intestinal recovery. Endpoints will be body weight, small intestinal (SI) weight, morphology, proliferative activity, and intestinal barrier function, explored in the acute and the recovery phase.

Results: Preliminary results have shown that the SI was significantly smaller in KO mice compared to WT littermates (4.2 ±0.2 vs. 4.8±0.2 % of BW, $p<0.05$). GLP-2 treatment did not affect the SI weight. We expect to have the majority of the remaining results ready by the time of the conference.

Conclusion: In conclusion, GLP-2R KO and WT mice enable us to investigate whether endogenous GLP-2 can protect and affect the recovery after intestinal injury.

PO 38

The link between GLP-1 and ANP explored in *ex vivo* perfused pig organs

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Introduction: An earlier report disclosed a link between atrial natriuretic peptide (ANP) and glucagon-like peptide-1 (GLP-1). However, controversy still persists; specifically whether this hormonal link also applies in larger animals including man. Our aim was to explore the link in ex vivo perfused pig organs. Experiments were performed in standard protocols for transplantation research. In the lung experiment, we used a protocol that was designed to explore the effect of GLP-1 in a model of 2 h of warm ischemia. In the heart experiment, we used a protocol designed to examine the best coronary artery flow in a portable heart perfusion system.

Methods: Anesthetized pigs (n=13) were given a bolus of i.v. liraglutide (40 µg/kg) or saline one hour before euthanization. Blood was collected at 0, 10 and 60 min. After 2 h of warm ischemia in situ, the lungs were excised and placed in the ex vivo lung perfusions (EVLP) machine (XVIVO); ventilated and perfused with autologous red blood cells and Steens solution. Samples were obtained at 0, 10, 20, 30, 40 and 60 min after adding 1.125 mg liraglutide or saline to the system.

Beating hearts (n=6) was placed in the heart perfusion system (Transmedics) and perfused with autologous whole blood and perfusion buffer. After a stabilization period of 20 min; GLP-1 (7-37) was infused for the next 60 min. Samples was obtained at -20, -10, 0, 5, 10, 15, 20, 30, 40, 60 and 80 min. Total proANP was measured in all plasma and perfusion samples using a validated and modified radioimmunoassay specific for porcine proANP.

Results: In the anesthetized pigs, liraglutide did not affect the plasma concentrations of proANP. The concentration of proANP in the perfusion buffer increased during the EVLP in both groups; however, the increase was faster and more pronounced in the liraglutide group. In the hearts, proANP concentrations were unaffected by GLP-1 infusion.

Conclusion: ProANP is produced and secreted from the isolated pig lungs. Furthermore, our data suggest that GLP-1 activation may potentiate the pulmonary secretion.

PO 39

Liraglutide treatment increases expression of atrial natriuretic peptide and decreases endothelin-1 expression in a mouse model of obstructive pulmonary disease

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Background: The gut hormone glucagon-like peptide-1 (GLP-1) improves lung function in a mouse model of chronic obstructive pulmonary disease (COPD) when administered in pharmacological doses. However, the mediators are unknown and it remains elusive whether endogenous GLP-1 also has protective functions. The purpose of this project is therefore to unravel both of the above. We hypothesize that GLP-1 causes smooth muscle in bronchioles and bronchi to relax, thereby reducing the airway obstruction and that this may be mediated via atrial natriuretic peptide (ANP) secreted either from the atrial cardiomyocytes or GLP-1R bearing cells in the lung.

Methods: In a combined model of asthma and COPD, mice were subjected to inhalations of aerosol ovalbumin and lipopolysaccharide. Animals were either treated with vehicle, GLP-1 receptor agonist (liraglutide (300 mg/kg*d)) or -antagonist (exendin 9-39 (10mg/kg*d)), and lung function was measured in a whole body plethysmograph using enhanced pause (PenH) as a parameter of bronchoconstriction. mRNA levels of ANP, ANP receptor A (ANPR-A) and Endothelin-1 (ET-1) was quantified to investigate potential changes in gene expression.

Results: Endogenous GLP-1 did not have a protective role in the pulmonary system, but consistent with previous observations, pharmaceutical dose of GLP-1 was protective (shown by significant lower PenH levels). ANP expression was up-regulated upon liraglutide treatment compared to the vehicle treated group ($p=0.0089$), whereas the ANP receptor (ANPR) was up-regulated in the vehicle group ($P<0.0001$). Furthermore, expression of ET-1 - a peptide regulating bronchoconstriction - was down-regulated by liraglutide compared to vehicle ($P<0.0001$).

Conclusion: Liraglutide treatment has a protective effect in a murine mouse model of COPD. Gene expression analysis indicates that this effect might be mediated via increased levels of ANP, which may have bronchodilating effects and/or via decreased levels of ET-1. The protective effects of GLP-1 on the pulmonary system warrants further studies and suggest that GLP-1 have an underappreciated role in lung physiology and pathophysiology, and may constitute a treatment strategy for COPD.

PO 40

The GLP-1 receptor agonist exenatide ameliorates bone composition and tissue material properties in high fat fed diabetic mice

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Type 2 diabetes mellitus (T2DM) has recently been recognized as a significant risk factor for bone fragility. Careful investigations of bone strength in human studies suggested possible alterations of bone composition, although this axis has poorly been investigated. The main aim of this study was to evaluate the impact of high fat diet-induced diabetes and therapy using the clinically approved GLP-1 receptor agonist, exenatide, on tissue bone strength and compositional parameters. Male NIH swiss mice had free access to high fat diet for 16 weeks to induce diabetes prior to commencement of the study. Exenatide was administered twice daily by i.p. injection at a dose of 25 nmol/kg for 52 days. Normal and high fat diet fed (HFD) mice injected with saline were used as controls. Bone strength was assessed at the organ level by 3-point bending and at the tissue level by nanoindentation. Bone microarchitecture was investigated by microcomputed tomography and bone composition was evaluated by Fourier transform infrared imaging. HFD mice exhibited profound alterations of bone strength at both the organ and tissue level. Collagen maturity as well as trabecular and cortical bone microarchitectures were abnormal in these animals. Administration of exenatide, led to clear ameliorations in bone strength at the organ and tissue levels by restoring cortical microarchitecture, osteoblast bone formation, collagen maturity and by reducing carbonate moieties in the hydroxyapatite bone mineral. These results bring new light on the mode of action of exenatide in bone physiology and demonstrate the value of GLP-1 mimetics in the treatment of fragility fractures in diabetes.

PO 41

Bone remodeling is tightly controlled by enteroendocrine K-cell products

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Introduction: The link between bone remodeling and gut hormones has been suggested through several studies. Among gut hormones, GIP seems to have an overriding role. GIP is mostly secreted by duodenal K-cells with xenin, somatostatin, cholecystokinin and secretin. The aim of this study is to investigate the role of secretory products of enteroendocrine K-cells in bone physiology.

Material and methods: We used K-cell deficient mice (GIP-DT), GIP knock out mice (GIP-GFP-KI) and mice injected with GIP, xenin, cholecystokinin, somatostatin analog (RC160) or secretin. We investigated bone strength, mass and quality by three-point bending, nanoindentation, microCT, FTIR and qBEI.

Results: Ablation of K-cells (GIP-DT) results in a normal bone phenotype, although GIP was absent. Ablation of GIP only (GIP-GFP-KI) led to significant alterations of cortical and trabecular bone represented by lower BV/TV (-28%) and Ct.Th (-13%). as well as bone strength. Bone matrix composition was also significantly modified with reduction in collagen maturity (-23%). To explain the discrepancy between these two models, we administrated stable analogs of K-cell products in normal mice. Only GIP- and xenin-injected mice exhibited significant differences in bone strength as compared with saline-injected mice. GIP and xenin also led to opposite trabecular bone microarchitecture alterations, as represented by higher bone mass with GIP (+22%) and lower bone mass with xenin (-13%). GIP and xenin also changed the organic phase of the bone matrix as represented by higher (+12%) and lower (-37%) enzymatic collagen crosslinking, respectively. None of the other gut hormone analogues did modify bone strength, mass or quality.

Conclusion: Our data highlight a previously unknown action of GIP and xenin, which act in concert to moderate gut-bone connectivity and shed new light on the control of bone remodeling by the gut.

PO 42

Glucagon-like peptide-1 and its cleavage products are renoprotective in murine diabetic nephropathy

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Aims: Incretin based therapies, including GLP-1 receptor agonists and DPP-4 inhibitors, are potent glucose lowering drugs. Still, only GLP-1 receptor agonists with close peptide homology to GLP-1 (liraglutide and semaglutide) but not exenatide based GLP-1 receptor agonists nor DPP-4 inhibitors were recently found to reduce cardiovascular events. This differential response might relate to GLP-1 receptor independent actions of GLP-1 cleavage products liberated by some agonists.

To test this hypothesis we directly compared metabolic, renal and cardiac effects of GLP-1 and its cleavage products in the diabetic environment of db/db mice.

Materials and Methods: Using an adeno-associated viral vector system we overexpressed DPP-4 resistant GLP-1(7-37Mut8) and the two GLP-1 cleavage products, GLP-1(9-37) and GLP-1(28-37) in diabetic db/db mice on high fat diet.

Results: Only GLP-1(7-37Mut8), but none of the cleavage products significantly improved glucose metabolism. Still, all GLP-1 constructs significantly reduced tubulointerstitial renal damage, lowered expression of the tubular injury markers and attenuated renal accumulation of macrophages and T cells. This was associated with a systemic immunomodulatory effect and reduced mortality in a combined analysis for all GLP-1 constructs.

Conclusion: GLP-1 and its cleavage products (9-37) and (28-37) showed potent renoprotective actions, which were most likely mediated by immunmodulatory, anti-inflammatory effects independent of the GLP-1 receptor and glucose metabolism.

PO 43

The fat sensing receptors GPR40 and GPR119 are decreased in the small intestine of mice after fat-self-administration

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Background and aim: Triacylglycerol (TAG) is the most abundant dietary lipid, and after consumption, TAG is being digested in the small intestine by various lipases resulting in 2-monoacylglycerol (2-MAG), and free fatty acids (FFA). These digestion products can bind to several receptors, e.g. GPR40 and GPR119. The GPR119 is a Gs coupled receptor, that upon binding of 2-MAG causes GLP-1 and GIP release. The long chain fatty acids can bind to the GPR40 receptor, a Gq coupled receptor, involved in CCK and GLP-1 release. In this study we wanted to investigate the effect of daily fat self-administration on the expression of the GPR40 and GPR119 receptors in the jejunum of C57BL6 mice.

Materials and methods: We used a behavior model, where food-restricted C57BL6 mice with implanted catheters into the stomach, were trained to self-administer an Intralipid 30 % emulsion for a one-hour daily session over a couple of months. We wanted to study whether a) the daily fat self-administration training could affect GPR40 and GPR119 expression relative to non-trained controls and b) whether acute voluntary fat intake affects the GPR40 and GPR119 receptor expression.

Results and conclusion: We found that the GPR40 expression in the jejunum was downregulated in both the pre- and post-session groups compared to the control group. This shows that the chronic fat intake or chronic food-restriction affects the GPR40 expression in the jejunum. For GPR119 we found a significant downregulation between the control group and the post-session group. Indicating that the acute voluntary fat intake effects the GPR119 expression in the jejunum, but that the daily self-administration training does not affect the GPR119 expression in the jejunum.

PO 44

Establishing the cellular makeup of the gut-brain axis by single-cell sequencing

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The gut-brain axis has been shown to mediate metabolic and neurological responses to the intake of nutrients. The cellular makeup and circuitry of the axis, however, is incompletely understood. We have addressed this by using single-cell sequencing to profile the three primary nodes that make up the axis: the gut, vagus nerve and brainstem. In the gut we have biased our sampling towards enteroendocrine cells (EECs) in the stomach, small and large intestines. In total we have profiled ~25,000 EECs and uncovered 19 distinct types in the mouse. Sequencing the same gut regions in the human has enabled us to confirm the translation of these findings and highlighted a few notable differences. Sequencing the mouse nodose ganglia, which contains the cell bodies of the afferent vagus nerve, has revealed ~20 cell types. Using tracing techniques, we have established the specific neuron types that innervate different regions of the gut. Finally, sequencing the brainstem has established the neuronal cell types that can receive input from the vagus and transmit the messages to different brain regions. Using bioinformatics, we have analyzed the expression of different secreted products and cognate receptors to infer potential cell signaling partners. In the future we aim to establish the circuits and harness them for therapeutics.

POSTER SESSION 6:
CLINICAL SCIENCE
GUT, BILE, AND BIHORMONAL RECEPTOR AGONISTS IN THE THERAPY OF DIABETES AND OBESITY

PO 45

Evidence of GIP-induced regulation of fatty acid desaturase 2 (FADS2) gene expression in subcutaneous adipose tissue

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Aims: GIP is important regulator of postprandial metabolism by stimulating insulin secretion and inhibiting lipolysis in the adipocytes. GIP is also involved in the development of the low-grade inflammation in the body upon high-fat and high-sucrose diet, but complete mechanisms of this action remained poorly understood. Fatty acid (FA) composition affects obesity-associated low-grade inflammation. Recently human studies shown that FADS2 gene polymorphism is associated with FA metabolism and adipose tissue (AT) inflammation. Here we aimed to investigate the effect on GIP infusion on FADS2 gene expression in subcutaneous adipose tissue and FA composition in humans.

Methods: Eleven male subjects without diabetes (age: 36,9 ± 1,9 years; body mass index 32,2 ± 1,0 kg/m2) underwent saline infusion (0.9% NaCl, Fresenius, Germany), GIP infusion at an infusion rate of 2.0 pmol kg−1min−1, lipid infusion or combined GIP and lipid infusion of 4 hours duration. Subcutaneous adipose tissue biopsies were taken at −40 min and 240 min of infusions and FADS2 mRNA levels were detected by real-time PCR. FADS2 desaturating activity was calculated as a ratio of 18:3n-6 to 18:2n-6 FA, 20:3n-6 to 20:2n-6 FA, 22:4n-5 to 22:4n-6 FA and C22:6n-3 to C22:5n-3 FA. FA compositions were measured in plasma at 0 and 240 minutes of each test by LCMS.

Results: FADS2 gene expression was increased after GIP, lipid and combined GIP and lipid infusion ($p<0.01$) compared to saline infusion. 18:3n-6 to 18:2n-6 FA ratio was significantly down-regulated at the end of GIP+lipid and lipid infusion, but no changes in the ratio under GIP infusion alone.

Conclusion: The observed GIP-dependent upregulation of FADS2 gene in adipose tissue may influence regulation of adipose tissue inflammation in postprandial state and in obesity.

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Effects of pioglitazone on glucose-dependent insulinotropic polypeptide-mediated insulin secretion and adipocyte receptor expression in patients with type 2 diabetes

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Incretin hormone dysregulation contributes to reduced insulin secretion and hyperglycemia in patients with Type 2 Diabetes Mellitus (T2DM). Resistance to glucose dependent insulinotropic polypeptide (GIP) action may occur through desensitization or down-regulation of β-cell GIP receptors (GIP-R). Studies in rodents and cell lines show GIP-R expression can be regulated through peroxisome proliferation-activated receptor-g (PPARg) response elements (PPRE). Whether this occurs in humans is unknown. To test this, we conducted a randomized, double-blind, placebo-controlled trial of pioglitazone therapy on GIP-mediated insulin secretion and adipocyte GIP-R expression in subjects with well controlled T2DM. Insulin sensitivity improved, but the insulinotropic effect of infused GIP was unchanged following 12 weeks of pioglitazone treatment. In parallel, we observed increased GIP-R mRNA expression in subcutaneous abdominal adipocytes from subjects treated with pioglitazone. Treatment of cultured human adipocytes with troglitazone increased PPARg binding to GIP-R PPRE. These results show PPARg agonists regulate GIP-R expression through PPRE in human adipocytes, but suggests this mechanism is not important for regulation of the insulinotropic effect of GIP in subjects with T2DM. Since GIP has anti-lipolytic and lipogenic effects in adipocytes and promotes adipogenesis, the increased GIP-R expression may mediate accretion of fat in patients with T2DM treated with PPARg agonists.

PO 47

Biliopancreatic diversion with duodenal switch (BPD-DS) and single-anastomosis duodeno-ileal bypass with sleeve gastrectomy (SADI-S) result in distinct post-prandial hormone profiles

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Biliopancreatic diversion with duodenal switch (BPD-DS) is the most effective bariatric intervention to treat morbid obesity and related disorders. Single-anastomosis duodeno-ileal bypass with sleeve gastrectomy (SADI-S) is a new bariatric procedure devised with the purpose of simplifying the complexity of the BPD-DS technique while retaining efficacy.

Previous studies reported that both surgeries result in similar rates of weight loss and overall metabolic improvement. However, how the underlying endocrine mechanisms involved in BPD-DS and SADI-S compare, is still unknown. Therefore, the purpose of this study was to assess the hormone response to a standardized mixed meal after BPD-DS or SADI-S.

Non-diabetic subjects submitted to BPD-DS (n=9) or SADI-S (n=9) on average 1.5 years earlier, underwent a liquid mixed-meal tolerance test (MMTT) to assess the baseline and post-prandial profile of glucose, enteropancreatic hormones and total bile acids.

Fasting glucose, enteropancreatic hormones and total bile acids levels after BPD-DS and SADI-S were similar. After the MMTT, the response of subjects who underwent SADI-S was characterized by higher glucose (t=30 min: $p<0.05$; iAUC: 156.1 ± 46.2 vs 103.4 ± 35.8 mmol/L x min, $p=0.02$), GLP-1(t=30 min: $p<0.05$; iAUC: 5388 ± 3010 vs 2959.0 ± 2146 pmol/L x min, $p=0.02$), glucagon (t=30 min: $p<0.05$; iAUC: 678.7 ± 295.2 vs 376.9 ± 215.7 pmol/L x min, $p=0.02$), insulin (t=30 and 45 min: $p<0.05$); and C-peptide levels (t=30 and 45 min: $p<0.05$), when compared to BPD-DS.

The post-prandial hormone secretion profiles of subjects who underwent SADI-S are characterized by higher levels of glucose, GLP-1, glucagon, insulin and C-peptide, when compared to BPD-DS. These data show that despite having apparently similar clinical outcomes, the endocrine mechanisms underlying the weight loss and metabolic effects of the two surgical procedures are potentially different.

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PO 48

Bile modulates secretion of incretins and insulin: A study on human extrahepatic cholestasis

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Changes in bile flow after bariatric surgery may beneficially modulate secretion of insulin and incretins leading to diabetes remission. However, the exact mechanism(s) involved are still unclear. We here propose an alternative method to investigate the relation between alterations in physiological bile flow and insulin and incretin secretion by studying the changes in gut-pancreatic function in extrahepatic cholestasis in non-diabetic humans. To pursue this aim, fifty-eight non-diabetic patients (mean age 57±7 yrs ±SE), with recent diagnosis of periampullary tumors, underwent an OGTT, and a subgroup of 16 patients also underwent 4-hour mixed meal tests and hyperinsulinemic euglycemic clamps. The analysis of the entire cohort revealed strong inverse

correlation between total bilirubin levels and insulinogenic index (r-0.37, p<0.01). When subjects were divided on the basis of bilirubin levels, used as marker of altered bile flow, subjects with high bilirubin levels displayed inferior glucose control (P=0.04) and decreased insulin secretion during OGTT (P<0.01), while no changes in whole-body insulin resistance were observed between groups.

Altered bile flow elicited a markedly greater increase in glucagon (N-Bil 5.62±1.08 vs H-Bil: 14.5± 2.21 pmol/L, P<0.01) and GLP-1 secretion (N-Bil: 19.5±2.89 vs L-Bil: 46.7± 10.9 pmol/L, P=0.02) at fasting state and following the meal both glucagon (P= 0.010) and GLP-1 (P=0.016) levels remained increased over time. Conversely, GIP levels were comparable at the fasting state (N-Bil: 10.3±2.87 vs H-Bil: 11.6± 3.87 pmol/L, P=0.02), while the increase following meal ingestion was significantly blunted in subjects with cholestasis (P = 0.013).

The analysis of the entire cohort revealed strong correlations, both in the basal levels (basal glucagon r= -0.78, P=0.01) and as AUC after the meal test (AUC glucagon 0-240 min r= -0.73, P=0.02). Further, we found a strong correlation between total bilirubin levels and basal GLP-1 levels (r= -0.40, p<0.01), while no correlation was found for the GLP-1 AUC after the meal test.

Our findings suggest that acute extrahepatic cholestasis determines a significant impairment in the entero-endocrine gut-pancreatic secretory function. The altered bile flow might determine a direct deleterious effect on beta cell function.

PO 49

Disclosing gut dynamics eliciting post-bariatric hypoglycemia

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Postprandial hypoglycemia (PPH) is a rare yet disturbing complication of bariatric surgery. PPH etiology is controversial, while management is empirical and remains clinically challenging. Our aim was to ascertain how intestinal hormone dynamics relate to post-bariatric PPH.

Nineteen glucose-tolerant patients long-submitted to RYGB underwent a standardized liquid meal mixed-meal tolerance test (MMTT) after an overnight fast. Blood sampling was performed at baseline and at timed intervals up to two hours for glucose, insulin, C-peptide, glucagon, GLP-1, GIP and PYY measurement. Insulinogenic index (IGI) was calculated as the ratio of incremental C-peptide from fasting to 30 minutes of the MMTT to glycemia excursion in the same time window and insulin secretion rate (ISR) was determined by deconvolution of C-peptide levels.

During the MMTT, 42% of the patients developed PPH (Hypo n=8 vs NoHypo n=11; glucose nadir <55mg/dL vs ≥55mg/dL). The two patient groups presented similar anthropometric (p=0.919) and clinical features both before and after RYGB. The main trigger for PPH was insulin secretion (p=0.013), predominantly meal-triggered (IGI: p=0.009) and non-incretin related (p>0.05). In addition, higher glucagon rise preceding glucose, insulin and C-peptide peaks, was correlated with a decreased risk for PPH (p=0.008).

PPH in post-RYGB patients is triggered by reactive hyperinsulinemia. The hormonal profile elicited after a MMTT provides relevant insights into the endocrine mechanism underlying PPH, which could ultimately be targeted to prevent or treat PPH.

Funding: The study was funded by FCT (UID/Multi/0215/2016).

PO 50

PYY plays a key role in the resolution of diabetes following bariatric surgery in humans

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Background: Bariatric surgery leads to early and long-lasting remission of type 2 diabetes (T2D). However, the mechanisms behind this phenomenon remain unclear. Among several factors, gut hormones are thought to be crucial mediators of this effect. Unlike GLP-1, the role of the hormone peptide tyrosine tyrosine (PYY) in bariatric surgery in humans has been limited to appetite regulation and its impact on pancreatic islet secretory function and glucose metabolism remains under-studied.

Methods and Findings: Here we have examined the changes in PYY concentrations six months after bariatric surgery in obese patients compared with healthy control subjects, and their effects on human islet functions. By means of an experimental paradigm using donor human pancreatic islets ex-vivo and sera from obese patients with T2D before and after bariatric surgery, we demonstrate that PYY is a key effector of the early recovery of impaired glucose-mediated insulin and glucagon secretion in bariatric surgery. We establish that the short chain fatty acid propionate and bile acids, which are elevated after surgery, can trigger PYY release not only from enteroendocrine cells but also from human pancreatic islets and that these metabolites can affect islet secretory function. In addition, we identify IL-22 as a new factor which is modulated by bariatric surgery in humans and which directly regulates PYY expression and release.

Interpretation: This study shows that the metabolic benefits of bariatric surgery can be emulated ex vivo. Our findings are expected to have a direct impact on the development of new non-surgical therapy for T2D correction.

PO 51

Continuous glucose monitoring reveals comprehensive glucose control with MEDI0382 in patients with type 2 diabetes mellitus

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Background: MEDI0382 is a balanced glucagon-like peptide-1/glucagon receptor dual agonist under development for providing glucose control and disease-modifying weight loss in type 2 diabetes mellitus (T2DM).

Methods: In this double-blind phase 2a study (NCT03244800), overweight or obese patients with T2DM were randomized to receive once-daily subcutaneous MEDI0382 or placebo for 49 days. Doses were up-titrated weekly (cohort 1) or every 2 weeks (cohort 2) from 50 µg to 300 µg. The co-primary endpoints (assessed in cohort 1) were percentage changes from baseline in glucose area under the curve ($AUC_{0–4h}$) after a mixed-meal tolerance test and body weight. Double-blind CGM was performed from day –2 to day 49 in cohort 1.

Results: There were significant reductions from baseline to the end of treatment in cohort 1 with MEDI0382 (n = 26) vs placebo (n = 13) in glucose $AUC_{0–4h}$ (least squares mean [90% confidence interval], –21.52% [–25.68, –17.37] vs 6.32% [0.45, 12.20]; $P < 0.001$) and body weight (–3.41% [–4.37, –2.44] vs –0.08% [–1.45, 1.28]; $P = 0.002$). CGM revealed rapid and consistent reductions in glucose levels with MEDI0382 therapy. Patients treated with MEDI0382 vs placebo spent a greater proportion of time within the target glycemic range (70–140 mg/dL [inclusive]; 67.9% vs. 43.2%; $P = 0.002$) and had lower 7-day daily average glucose levels (118.8–133.2 mg/dL vs 153.0–154.8 mg/dL).

Conclusions: MEDI0382 stabilized glucose levels and elicited sustained weight loss in overweight or obese patients with T2DM. CGM revealed 24-hour glucose lowering with MEDI0382.

PO 52

MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, significantly reduces hepatic fat content in subjects with type 2 diabetes mellitus

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Background: MEDI0382 is a balanced glucagon-like peptide 1 (GLP-1)/glucagon receptor dual agonist in development for type 2 diabetes mellitus and nonalcoholic steatohepatitis. We investigated the effects of MEDI0382 on hepatic fat content in overweight/obese subjects with type 2 diabetes and a body mass index of 27–40 kg/m^2.

Methods: In a phase 2a study (NCT02548585), 51 subjects were randomized to receive MEDI0382 200 µg or placebo daily during a 41-day treatment period. Quantification of liver proton density fat fraction, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) was performed as an exploratory analysis in a subset of patients (MEDI0382, **n** = 17; placebo, *n* = 21) who had magnetic resonance imaging scans taken at baseline and on day 41.

Results: Mean ± SD liver fat content at baseline was 15.74 ± 8.97% and 18.07 ± 8.38% in the MEDI0382 and placebo groups, respectively. Patients treated with MEDI0382 showed a highly significant relative reduction from baseline in hepatic fat content versus placebo. This was positively correlated with reductions in both body weight (r = 0.49, P = 0.002) and alanine aminotransferase (r = 0.42, P = 0.009). A significant decrease in liver volume with MEDI0382 vs placebo was also observed. Treatment with MEDI0382 was also associated with reductions in both SAT and VAT versus placebo.

Conclusion: These data demonstrate the efficacy of MEDI0382 in reducing liver fat and its potential for the treatment of nonalcoholic fatty liver disease, including steatohepatitis.

PO 53

GLP-1-mediated delivery of thyroid hormone T3 reverses diet-induced obesity and glucose intolerance in mice

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Helmholtz Center Munich

POSTER SESSION 7:
CLINICAL SCIENCE
GLP-1 RECEPTOR AGONISTS AND GLUCAGON

PO 54

Effects of GLP-1R agonists on beta cell survival, function and granule motility

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Beta cell dysfunction is crucial to the onset and progression of type 2 diabetes (T2D). We evaluated the effects of GLP-1R agonists on the ultrastructure, function and granule mobility of human pancreatic islets (HI) from non-diabetic (ND; n: 15, 9M/6F; age: 41-81 yrs) and T2D (n: 8; 4M/4F; age: 64-83 yrs) organ donors.

Non-diabetic HI were exposed for 48h to 16.7 mM glucose (HG) or 0.5 mM palmitate (PALM) with or without 1.0, 10 or 100 nM GLP-1. Similarly, T2D islets were cultured for 48h in presence of increasing concentrations of GLP-1. Then, beta cell ultrastructure and function were evaluated by electron microscopy (EM) and batch static incubation, respectively. In addition, dissociated HI were transfected with Syncollin-EGFP in order to fluorescently mark the granules, and then exposed to control medium, PALM or PALM + 10 nM exendin-4. By advanced imaging confocal microscopy-based (iMSD), the granule average diffusion law and parameters describing granules motility [i.e. the local and the anomalous (alpha) diffusion coefficients] were defined and applied.

EM (>1,000 endocrine cells counted) showed that the gluco- or lipotoxic conditions significantly increased the amount of beta cells with signs of apoptosis and decreased the volume density of the insulin granules, which was prevented by GLP-1. Glucose-stimulated insulin release, that was reduced ($p<0.05$) in islets exposed to HG (insulin stimulation index, ISI, from 3.3±0.8 to 1.8±0.3) or PALM (ISI from 3.0±0.6 to 2.1±0.5), was preserved by concomitant exposure to 10 or 100 nM GLP-1. In T2D islets, GLP-1 treatment reduced ($p<0.05$) apoptotic beta cell proportion (from 3.7±0.5 to 2.1±0.7%) and ameliorated ($p<0.05$) insulin secretion (ISI from 1.6±0.4 to 2.2±0.3). Finally, by iMSD, it was shown that PALM exposure reduced granule directionality in response to an acute glucose stimulation; the presence of exendin-4 was able to normalize alpha values.

GLP-1R agonists were confirmed to have several beneficial effects on ND and T2D beta cell survival and function, including a better insulin granule motility.

Supported by DRINN

PO 55

Combination of GLP-1 receptor agonist (GLP-1RA) treatment and physical activity for maintenance of diet-induced weight loss and metabolic health

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Background: The gut hormone glucagon-like peptide-1 (GLP-1) stimulates insulin secretion, reduces appetite and food intake. Therefore, GLP-1RAs such as liraglutide are used in treatment of type 2 diabetes and obesity. On the other hand, regular physical activity is an important lifestyle component for weight loss maintenance and metabolic health.

Aim: To investigate the combination of liraglutide treatment and regular physical activity on long-term weight loss maintenance and health outcomes.

Methods: We have recruited 223 obese men and women (BMI 32-43 kg/m2, age 18-65 years). Initially, participants undergo an 8-week very-low-calorie diet (800 kcal/day) to lose at least 5% of body weight. Subsequently, participants are randomized to one of four study arms: 52 weeks with 1) liraglutide 3 mg/day, 2) placebo, 3) exercise 150 min/week + placebo, and 4) exercise 150 min/week + liraglutide 3 mg/day. Primary endpoints are weight and body composition (DEXA scan) and secondary endpoint is metabolic health. Explorative objectives include appetite regulation (meal test), physical fitness (peak oxygen test, strength, and stair climb test), vascular endothelial function, immunometabolic profile of adipose tissue, food preferences and appetite sensation. Furthermore, faeces, urine, saliva and sperm cells are collected.

Preliminary data analysis: 174 participants (63% females and mean age 42) have completed the initial 8-weeks weight loss phase resulting in mean weight loss of 13kg (95% CI: 12.6, 13.3; $p<0.001$) equivalent to a 12% body weight loss. This weight loss was accompanied by decreased fasting plasma glucose (7%), blood pressure (8%), plasma cholesterol (18%), and plasma triglycerides (28%) ($p<0.001$ for all analyses).

Conclusion: The present preliminary results illustrate that 8 weeks with a very-low-calorie diet was effective resulting in a major weight loss of 12% of body weight accompanied with beneficial effects on plasma glucose, blood pressure and plasma lipids.

PO 56

The glucagon-like peptide-1 receptor agonist lixisenatide reduces postprandial glucose excursions in totally pancreatectomised patients

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Background and aims: The extrapancreatic effects of glucagon-like peptide-1 (GLP-1) have been difficult to disentangle. Treatment of diabetes secondary to total pancreatectomy remains a challenge. We investigated the effects of the GLP-1 receptor agonist, lixisenatide, on postprandial glucose metabolism in totally pancreatectomised patients.

Materials and methods: In a double-blinded, randomised, cross-over study, we recruited 12 totally pancreatectomised patients (3 women; age: 65.0±9.5 [mean±SD] years; BMI: 22.9±3.9 kg/m2) and 12 control subjects (4 women; age 64.4±7.6 years; BMI: 24.0±2.9 kg/m2). Both groups were examined during two 3-hour liquid mixed meal tests (with 1.5 g paracetamol for evaluation of gastric emptying) after single-dose injection of 20 µg of lixisenatide or placebo, respectively. Patients received their regular dose of basal insulin the night before each experimental day; no insulin was given during the meal tests. Blood was sampled for measurements of plasma/serum concentrations of glucose, glucagon, C-peptide and paracetamol.

Results: Compared to placebo, lixisenatide significantly reduced postprandial plasma glucose excursions in totally pancreatectomised patients (AUC±SEM: 2,715±179 vs 3,473±177 mmol/l × min, p=0.006) and controls (AUC: 814±14 vs 1,152±57 mmol/l × min, p<0.0001) (figure 1). In totally pancreatectomised patients, C-peptide was undetectable in plasma and lixisenatide significantly reduced gastric emptying as well as postprandial glucagon responses (AUC: 620±162 vs 1,479±271 pmol/l × min, p=0.003). In the control subjects, lixisenatide reduced postprandial plasma C-peptide responses (AUC: 105±19 vs. 261±34 nmol/l × min, p=<0.001) and decelerated gastric emptying significantly whereas postprandial glucagon responses were unaffected (AUC: 1,283±159 vs 1,253±225 pmol/l × min, p=0.879).

Conclusion: The GLP-1 receptor agonist lixisenatide reduces postprandial plasma glucose excursions in totally pancreatectomised patients. The mode of action seems to involve deceleration of gastric emptying and reduced postprandial responses of gut-derived glucagon.

PO 57

Levels of GIP, GLP-1, insulin and glucagon in type 2 diabetes and healthy subjects after a mixed meal tolerance test

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Introduction: Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone released by the K cells in the small intestinal mucosa upon food intake. The most pronounced function of the incretins (GIP and glucagon-like peptide-1 (GLP-1)) is to increase the insulin secretion from the b-cells in the pancreas. In addition, GIP receptors are also located in the brain, adrenal glands and adipose tissue and GIP has been found to be increased in obesity. Diabetes mellitus type 2 (T2DM) is associated with altered production and release of metabolic hormones, and the interplay between different hormones can be studied during a mixed-meal tolerance testing (MMTT).

To expand the available metabolic hormone immunoassays, a Total GIP ELISA has recently been developed by Mercodia. Since before, the Mercodia insulin ELISA is a well-established and robust assay often used to determine insulin release, and assays for intact glucagon (1-29) and total GLP-1 have been established and validated according to CLSI and FDA guidelines.

Methods: In the present study, we sought to investigate how preprandial and postprandial levels of insulin, glucagon, GIP and GLP-1 in plasma are affected by an MMTT, using the thoroughly characterized Mercodia assays. MMTT was carried out in cohorts of healthy individuals, and volunteers diagnosed with T2DM.

Sampling was performed preprandially and 30 and 120 minutes after the individual had begun consuming the meal.

Results: Preprandial GIP levels did not differ between the groups, but individuals with T2DM exhibited elevated preprandial levels of GLP-1, glucagon and insulin ($[GLP]_{Pre}$=7.4 pM, $[Gcg]_{Pre}$=11 pM, $[Ins]_{Pre}$=10 pM) compared to healthy controls ($[GLP]_{Pre}$=4.6 pM, $[Gcg]_{Pre}$=6.9 pM, $[Ins]_{Pre}$=4.6 pM). For healthy individuals, GIP levels decreased between 30 min and 120 min postprandially ($[GIP]_{30}$=105 vs $[GIP]_{120}$=85 pM), while for the T2DM group the mean concentration did not decrease ($[GIP]_{30}$=96 vs $[GIP]_{120}$=98 pM).

Moreover, T2DM was associated with a sustained insulin release, with higher insulin levels detected at 120 minutes compared with 30 minutes ($[Ins]_{30}$=29 vs $[Ins]_{120}$=39 pM), in contrast to healthy controls where the insulin concentrations peak after 30 minutes($[Ins]_{30}$=38 vs $[Ins]_{120}$=14 pM).

Conclusions: We conclude that the Mercodia ELISAs for the incretins GIP and GLP-1 and islet hormones insulin and glucagon performs with high sensitivity and specificity, when determining the levels of these hormones under physiological conditions, both in healthy and diabetic subjects.

PO 58

Incretin-based treatment: Glucose threshold for glucagon counter-regulation during hypoglycemia

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Incretin-based treatment is associated with low risk of hypoglycemia in type 2 diabetes (T2D) possibly due to its glucose-dependency in stimulating insulin secretion and a sustained glucagon response during hypoglycemia. This is of special relevance when it is combined with insulin or given to elderly patients since both insulin therapy and high age are associated with impaired glucagon counter-regulation. Incretin-based therapy may also be used as add-on to insulin therapy in type 1 diabetes (T1D), where there is also an impaired glucagon counter-regulation. The studies evaluated the glucagon counter-regulation to insulin induced hypoglycemia during incretin therapy in 4 different patient populations: vildagliptin (DPP-4 inhibition) + insulin in T1D (VILDA1), vildagliptin + insulin (±oral medication) in T2D (VILDA2), lixisenatide (GLP-1 receptor agonist) + basal insulin + metformin in T2D (LIXI) and sitagliptin (DPP-4 inhibition)+ metformin in elderly with T2D (SITA-CLAMP).

The studies were single-center, double-blind, randomized, placebo (PBO)-controlled crossover studies involving 18-29 subjects in each study with mean age 30 (T1D) 55, 59, 74 (T2D) yrs respectively, diabetes duration 9-14 yrs, baseline HbA1c 52-61 mmol/mol and BMI 25-33 kg/m2. Subjects received the study medication or placebo as add-on therapy for 4 weeks (6 weeks in LIXI) in random order with a four-week washout in-between. After each treatment period, the subjects underwent a hyperinsulinemic hypoglycemic clamp with targeting glucose at 2.5 mmol/l (VILDA1), 2.6 mmol/l (VILDA2), 3.5 and 2.8 mmol/l (LIXI), 3.5 and 3.1mmol/l (SITA-CLAMP); targeted glucose levels were preserved for 30 min.

Results showed that at 3.1 mmol/l and below, the glucagon responses were similar during incretin therapy as during placebo, whereas at 3.5 mmol/l (LIXI and SITA-CLAMP studies), the glucagon response was lower during incretin therapy than during placebo.

We conclude that 1) the glucose threshold for glucagon counter-regulation is between 3.5 and 3.1 mmol/l during incretin therapy, 2) the glucagon response to hypoglycemia is sustained during incretin therapy in all study populations and, therefore, 3) this may contribute to the low risk of hypoglycemia during incretin-based treatment also in susceptible and fragile patient groups.

PO 59

Intravenous arginine has no effect on the secretion of gut-derived glucagon in totally pancreatectomized subjects

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Background and aims: We recently provided evidence that glucagon also is secreted from extrapancreatic tissues in totally pancreatectomized (PX) patients - most likely from enteroendocrine L cells. The regulation of gut-derived glucagon secretion is unknown. In order to establish whether arginine (a strong stimulus for pancreatic glucagon secretion) stimulates gut-derived glucagon secretion, we performed an intravenous (iv) arginine test in PX patients and non-diabetic control subjects (CTRL) and employed a recently validated and highly sensitive and specific glucagon assay.

Materials and methods: Plasma glucagon and glicentin (a product of the enteroendocrine L cell) concentrations were measured at baseline (in the fasting state) and at 2, 5, 10, 15 and 30 minutes following iv infusion of 5 grams of arginine (administered over 5 minutes) in 12 PX patients (age [mean±SEM] 65±2.7 years; BMI 22.9±1.1 kg/m^2) and 12 matched CTRL (age 64.4±2.4 years; BMI 24.0±0.8 kg/m^2).

Results: PX patients exhibited significantly lower fasting plasma glucagon compared to CTRL (2.5±0.4 vs. 8.2±0.9 pmol/l, P<0.0001). In the CTRL group we observed an abrupt and significantly larger glucagon response following iv arginine (baseline-subtracted area under the curve (bsAUC) 347±32 pmol/l × min) compared to the PX patients (P<0.001) in whom no change in plasma glucagon was observed (bsAUC 0±7 pmol/l×min). In the CTRL group, no glicentin response was found (bsAUC -43±32 pmol/l×min), whereas a small response was observed in the PX group (136±70 pmol/l×min) (P<0.0001).

Conclusion: Iv infusion of arginine, often used to evaluate maximal pancreatic glucagon secretion, has no effect on gut-derived glucagon secretion in totally pancreatectomized patients.

PO 60

Intestinal glucagon and its role in diabetic hyperglycaemia

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Patients with type 2 diabetes are characterized not only by insufficient insulin secretion, but also by increased plasma glucagon concentrations, which contribute substantially to type 2 diabetic hyperglycaemia. Glucagon is considered a pancreas-derived hormone, but it has also been shown to be produced in the small intestine of pancreatectomized individuals and after gastric bypass surgery. Whether gut-derived glucagon contributes to the hyperglycaemia that characterizes type 2 diabetes remains unknown.

Using the perfused small intestine of mice (male, n= 4), we found an increased secretion of glucagon during luminal glucose stimulation in diet-induced obese mice (DIO) compared to wild type mice, whereas GLP-1 secretion was similar. Inspired by these early data, we will further identify and characterize intestinal glucagon in different disease models, and explore the extent to which it contributes to diabetic hyperglycaemia. We will use five animal models of disease: diabetic mice (DB/DB), DIO mice, genetically obese mice (OB/OB), and FATZO mice. First, we will investigate if enteroendocrine L cells from the small intestine in these models secrete glucagon, and confirm the molecular nature of the secreted glucagon using mass spectrometry. Subsequently, we will compare glucagon and glucose responses during oral administration and intraperitoneal injection of glucose, and evaluate the relative importance of gut-derived versus pancreatic-derived glucagon

during simultaneous administration of a glucagon receptor antagonist (Novo Nordisk 25-2648, 100 mg/kg) and saline, respectively. Finally, we will assess intestinal traces of glucagon in obese patients using ELISAs, a new monoclonal C-terminal antibody against glucagon, and mass spectrometry.

Identifying the underlying mechanisms of intestinal glucagon may provide new insights into the pathophysiology of type 2 diabetes and uncover potential new drug targets.

PO 61

Comparing the effect of three different DPP-4 inhibitors during 24 hours Cross-over study in metformin-treated type 2 diabetes individuals

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The extent of the glucose-reducing effect over the entire day after DPP-4 inhibition and whether different DPP-4 inhibitors (DPP-4i) differ are not known. We therefore compared the effect of three different DPP-4i (sitagliptin (SITA), vildagliptin (VILDA), saxagliptin (SAXA)) versus placebo (PBO) on glucose, insulin and incretin hormone levels over an entire day with standardized meal ingestion (breakfast, lunch and dinner) in well controlled T2D subjects.

After an overnight fast, twenty-four metformin-treated T2D subjects (12 male, 12 female, mean age 63 yrs, BMI 31.0 kg/m2, HbA1c 44.7mmol/mol=6%, diabetes duration 4 yrs. Stabil metformin dose ~1000mg) underwent four tests in random order: SITA; 100mg, VILDA; 50mg (twice a day), SAXA; 10mg, or PBO was given followed by ingestion of standardized breakfast (525kcal), lunch (780kcal) and dinner (560kcal) at specific time points. Blood samples were taken for analysis of glucose, insulin and incretin hormones; suprabasal 180 min areas under the curve (AUC) were calculated.

SITA, VILDA, and SAXA similarly suppressed the rise in glucose levels after all three meals. The AUCglucose180min were reduced by 29, 41 and 22% (SITA), 24, 34 and 24% (VILDA) and 22, 25 and 16% (SAXA) after breakfast, lunch and dinner respectively (at least $P<0.05$) compared to PBO. Insulin levels increased similarly after each meal ingestion in all four tests. Levels of intact GLP-1 and GIP increased similarly after all DPP4i ($P<0.001$). Levels of total GIP were lower after after DPP4i than after PBO after breakfast ($p<0.001$), but not after lunch or dinner. Based on these data we conclude that in metformin-treated and well controlled T2D subjects DPP-4i reduce glucose levels, sustain insulin levels and raise intact GLP-1 levels after breakfast, lunch and dinner with no significant difference between the three tested DPP-4i.

Trial ID: EudraCT 2013-005570-22

PO 62

Incretin mimetics show neuroprotective effects in neurological disorders

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The GLP-1 mimetic exendin-4 has shown good neuroprotective effects in several animal models of Parkinson's disease (PD). A pilot study testing exendin-4 (Byetta) in patients showed good protective effects in motor and cognitive tests. In this open-label trial, patients received the drug for 12 months. In a follow-up phase II trial that was double blind and placebo-controlled, the once-weekly formulation of exendin-4 (Bydureon) showed good protective effects. These impressive clinical effects are a proof of principle that demonstrate the neuroprotective properties of incretin analogues. The GLP-1 mimetic liraglutide also showed first encouraging results in pilot studies in people with Alzheimer's disease (AD) or with depression.

Exendin-4 is a first- generation GLP-1 mimetic that since has been superseded by superior GLP-1 receptor agonists. We have tested GLP-1 mimetics such as liraglutide and lixisenatide and found them to be superior to exendin-4 in the MPTP mouse model of Parkinson's disease. In addition, we tested GIP analogues that can cross the blood-brain barrier and showed good neuroprotective effects.

Novel dual GIP / GLP-1 receptor agonists have been developed to treat type II diabetes. We have tested several novel dual agonists and found superior neuroprotective effects in several animal models of PD, stroke or AD. When comparing the novel dual GIP / GLP-1 receptor agonists with single GLP-1 or GIP receptor agonists in animal models of PD, they showed superior protective effects.

Two clinical phase II trials testing liraglutide or lixisenatide in patients with PD are currently in progress, and a clinical phase II testing liraglutide in patients with AD is also ongoing. Other trials testing a novel dual agonist are in planning. The results obtained in preclinical studies are encouraging and suggest that these novel dual agonists may be capable of stopping progressive neurodegenerative disorders such as AD or PD.

POSTER SESSION 8:
CLINICAL SCIENCE
INCRETIN-BASED THERAPIES: GLP-1 RECEPTOR AGONISTS AND DPP-4 INHIBITORS

PO 63

Genetic variations of the GLP-1 receptor; basic pharmacological characterization to prepare for future personalized medicine for metabolic diseases.

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Backgrounds and aims: The GLP-1 receptor (GLP-1R) plays an important role for glucose-dependent insulin secretion. It also plays an important role in other metabolic functions and a dysfunctional GLP-1R may be involved in the development of metabolic disease. The aim of this study is to determine ligand binding, receptor signaling and recycling of naturally occurring GLP-1R variants identified in people with either type 2 diabetes (T2D) or in obese children in the Danish population, to investigate whether any of these mutations could be linked to metabolic dysfunction.

Materials and methods: 38 mutations spanning the whole receptor from the N-terminal domain, through the 7 transmembrane domains and the C-terminal domain were selected. The mutations were introduced in the human GLP-1R using quick-change PCR and confirmed by sequence analysis. Functional analyses: cAMP accumulation (using the DiscoverX developed system HitHunter®), β-arrestin recruitment (using the DiscoverX developed system PathHunter®) and real-time internalization (using a SNAP-tagged GLP-1R in the Tag-lite® system developed by Cisbio) were (or will be) performed, together with competition binding analysis.

Results: Initial data from the cAMP assays shows a variety of signaling outcomes, with some mutations being dead, some less active and some even more active compared to wt GLP-1R. These results create a fundament for in vivo phenotypical characterization of people carrying the mutations; studies that will be performed after finalizing the functional tests (arrestin recruitment, internalization and competition binding).

Conclusion: This project reveals a great variety of signaling efficiencies of the naturally occurring GLP-1R variants in these cohorts. It will pave the way for a deeper understanding of the role of the GLP-1 system in metabolic disease, and for future personalized treatment and classification of obesity and type 2 diabetes patients focusing on the GLP-1R.

PO 64

Safety effect of combination therapy with Liraglutide plus metformin on HGP and HbA_{1c} vs each therapy alone in patients with T2DM

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Background: Main hormons involved in regulation of glucose are glucagon like peptid GLP-1 and glucose dependent insulotropic polipeptid GIP. Both of them are inactivated by the enzim dipeptil peptidasa -4(DPP-4). Incretin based therapies include GLP-1 receptor agonists and DPP-4 inhibitors wich main actions are to increase secretion of insulin and inhibit secretion of glucagon.

Objectives: SGLT2 inhibitors cause an increase in HGP accompanied with an increase in plasma glucagon concentration. Increase in plasma glucagon concentration is partly responsible for the increase in HGP. The aim of study was to examine whether inhibition of glucagon secretion by liraglutide can prevent the increase in HGP.

Research and methods: 51 T2DM patients (age 55±1 years; 45% female; BMI=35,6±0,9; diabetes duration =7,8±0,9 years; FPG =7.5±0,7; HbA_{1C}=8,5±0,1%) were randomised to receive for 14 weeks (i) metformin 1g; (ii) liraglutide1,8mg or (iii) metformin1g plus liraglutide 1,8 mg. HGP (measured with 3-3 glucose infusion) and plasma glucagon concentration were measured before and after 14 weeks of treatment.

Results: Metformin monotherapy caused a significant reduction in HbA1c (-1,1±0,2%,p<0,01 and reduction in HGP by 15 %;Liraglutide monotherapy caused a 1,6±0,5 %, (P<0,01) reduction in HbA_{1C} accompanied by small 6% reduction in HGP without significant change in fasting plasma glucagon concentration. The combination of metformin plus liraglutide caused a greater reduction in HbA_{1C} (1,9±0,5%, p<0,05 vs met and liraglutide) and attenuated the increase in fasting plasma glucagon and basal HGP at 14 weeks.

Conclusion: Inhibition of glucagon secretion by SGLT2 can prevent the increase in HGP.

PO 65

Liraglutide administration improves hormonal/metabolic profile and reproductive features in women with HAIR-AN syndrome.

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Background: HAIR-AN syndrome, the coexistence of Hirsutism, Insulin Resistance (IR) and Acanthosis Nigricans, is a rare syndrome, characterized from clinical and biochemical hyperandrogenism accompanied with severe insulin resistance, chronic anovulation and metabolic abnormalities. Literally, HAIR-AN represents an extreme case of polycystic ovary syndrome (PCOS). In everyday practice the management of HAIR-AN constitutes a therapeutic challenge with the available pharmaceutical agents. Specifically, the degree of IR cannot be significantly ameliorated with metformin administration, whereas oral contraceptives chronic administration is associated with worsening of metabolic profile. Liraglutide, a GLP-1 analogue has been introduced with great success in the management of type 2 diabetes and exenatide, another agent of the same class has recently shown a significant improvement of hormonal/metabolic profile in significantly obese women with PCOS.

Aim of the study: To evaluate the impact of Liraglutide on hormonal /metabolic profile and ovarian function in women with HAIR-AN.

Subjects: Five women with the syndrome were studied for 6 months.

Setting: Outpatient clinic.

Results: In all participants a significant improvement regarding the degree of IR, androgen levels and the pattern of menstrual cycle was observed, accompanied with modest weight loss. Furthermore, one woman became pregnant during Liraglutide treatment giving birth to a healthy child.

Conclusions: Liraglutide, seems to be an effective alternative in the management of women with HAIR-AN.

PO 66

Effects of MEDI0382, a glucagon-like peptide 1/glucagon receptor dual agonist, on amino acids, ketones, and free fatty acids

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Background: MEDI0382 is a balanced glucagon-like peptide-1/glucagon receptor dual agonist under development for type 2 diabetes mellitus (T2DM). GLP-1 has been shown to promote glucose-dependent insulin release, delay gastric emptying, and suppress appetite. Glucagon has similar effects on appetite and gastric emptying and enhances energy expenditure by upregulating energy-expensive metabolic processes such as gluconeogenesis and fatty acid oxidation.

Methods: As an exploratory component to a double-blind, phase 2a study, fasting amino acid, beta-hydroxybutyrate, and free fatty acid levels were measured in overweight or obese patients with T2DM randomized to receive once-daily subcutaneous MEDI0382 (*n* = 26) or placebo (*n* = 13) for 49 days. Doses

were up-titrated every week from 50 µg to a maintenance dose of 300 µg, and measurements were performed at baseline and after 49 days of dosing.

Results: A significant decrease from baseline to day 49 with MEDI0382 vs placebo in alanine was observed (–0.5 vs 0.4 mg/dL; *P* = 0.017). Numerical reductions in other amino acid levels, including glutamate, cystine, valine, lysine, and tyrosine, were also seen. No clinically or statistically significant changes from baseline to day 49 were observed between treatment groups in beta-hydroxybutyrate or free fatty acid levels.

Conclusion: Despite the known effects of glucagon on fatty acid oxidation, MEDI0382 had no effect on ketogenesis or free fatty acids. Although the reason for this is unclear, enhanced insulin secretion may have counter-regulated this effect. MEDI0382 did, however, promote reductions in key glucogenic amino acids, and it is plausible that this could be related to increased gluconeogenesis and therefore glucagon receptor engagement. Indeed, similar patterns of amino acid reduction have been observed after bariatric surgery and have been attributed to excess circulating glucagon. We postulate that upregulation of an energy-consuming process such as gluconeogenesis could contribute to significant weight loss in subjects dosed with MEDI0382.

PO 67

Efficacy and safety of continuing sitagliptin when initiating insulin therapy in subjects with type 2 diabetes mellitus

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Background and aims: DPP-4 inhibitors (DPP4is) are often discontinued with initiation of insulin therapy but the impact of this discontinuation on efficacy and hypoglycemia has not been studied. In this double-blind trial the safety and efficacy of initiating insulin while continuing sitagliptin (SITA) were evaluated.

Materials and methods: Eligible patients had inadequately controlled T2DM on metformin (MET, ≥ 1500 mg/day) in dual or triple combination therapy with a DPP-4i and/or a sulfonylurea. Those on MET + SITA (100 mg/day) directly entered the trial; all others were switched to MET + SITA and stabilized during a run-in period. Subjects were randomized to continuing SITA or discontinuing SITA and switching to matching placebo, with both groups initiating insulin (LANTUS®), which was titrated based on fasting glucose.

Results: 746 subjects (mean HbA1c = 72.6 mmol/mol [8.8%], mean disease duration of 10.6 years) were randomized. After 30 weeks, continuing SITA was superior to discontinuing SITA in reducing HbA1c (p<0.001). Patients who continued SITA had a lower event rate of documented symptomatic hypoglycemia (blood glucose ≤3.9 mmol/L) and daily insulin dose compared to patients who discontinued SITA. Summary adverse event measures and change in body weight (Week 30) were similar in the 2 treatment groups.

Conclusion: With the initiation of insulin therapy, continuation of SITA resulted in superior glycemic efficacy and less documented symptomatic hypoglycemia.

PO 68

Safety and efficacy of sitagliptin compared with dapagliflozin in patients with T2D, mild renal impairment, and inadequate glycemic control on metformin +/- a sulfonylurea

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Background and aims: While choice of AHAs may be modified in patients with T2D and moderate or severe renal insufficiency, this is generally not the case in patients with mild renal insufficiency. Clinical trial data focused on this population, which represents ~40% of patients with T2D, are lacking. In a randomized, double-blind, active comparator-controlled clinical trial, the safety and efficacy of adding sitagliptin (SITA) (100 mg qd) or dapagliflozin (DAPA) (10 mg qd) to treatment of patients with eGFR ≥60 and <90 mL/min/1.73 m2 and HbA1c ≥53 and ≤80 mmol/mol (≥7.0% and ≤9.5%) while on metformin ± a sulfonylurea were assessed.

Materials and methods: The primary efficacy endpoint was change from baseline HbA1c at Week 24 (analyzed with a constrained longitudinal data analysis model), with a primary hypothesis of non-inferiority of SITA to DAPA based on the prespecified criterion of the upper bound of the between-treatment difference 95% CI (SITA minus DAPA) <0.3%; if the upper bound was <0.0%, SITA would be declared superior.

Results: Treatment groups were well balanced at baseline (n = 307 and 306, mean HbA1c, mmol/mol, [%] = 60.9 [7.7] and 61.2 [7.8], mean eGFR, mL/min/1.73 m2 = 79.4 and 76.9 for SITA and DAPA, respectively). At Week 24, LS mean changes from baseline HbA1c, mmol/mol (%) were -5.6 (-0.5) (SITA) and -3.9 (-0.4) (DAPA); between-group difference (95%CI) = - 1.7 (-2.9, -0.5) (-0.2 [-0.3, -0.0]), p=0.006, confirming both non-inferiority and superiority of SITA vs. DAPA. The pre-specified analysis of 2hr post-prandial glycemic excursion showed no significant difference between groups. The HbA1c goal of <53 mmol/mol (<7%) was met by 43% (SITA) and 27% (DAPA) of patients. Treatments were well tolerated; there were significantly fewer patients with drug-related adverse events (AEs) with SITA than with DAPA, but summary AE profiles were otherwise similar.

Conclusion: SITA treatment over 24 weeks resulted in greater glycemic efficacy and greater % of patients at HbA1c goal than DAPA in patients with T2D and mild renal impairment who were inadequately controlled on metformin ± a sulfonylurea.

PO 69

Effects of DPP-4 inhibitors, alone or in combination with pioglitazone, on palmitate-induced apoptosis and autophagy in human cardiac progenitor cells from control and diabetic subjects

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Physiological tissue turnover in the heart requires the recruitment of functional multipotent cardiac progenitor cells (CPCs). A defective CPC compartment, in terms of CPC number and pro-angiogenic capacity, contributes to diabetes- and hyperglycemia-related heart failure in humans. GLP-1-based therapies, including the DPP-4 inhibitors, improve cardiac function in vivo and ameliorate myocardial and endothelial dysfunction in vitro. Pioglitazone has also demonstrated pleiotropic anti-oxidant and anti-atherogenic effects. The aim of this study was to evaluate the effects of alogliptin and pioglitazone, alone or in combination, on the viability of human CPCs from non-diabetic and type 2 diabetic (T2D) individuals challenged with saturated fatty acids. Human CPCs were isolated from auricle biopsies obtained during cardiac surgery for CABG and/or valve surgery. In the CPCs isolated from control subjects, both alogliptin (10 μM) and pioglitazone (10 μM) stimulated Akt ($p<0.05$) but not Erk phosphorylation. By contrast, neither alogliptin nor pioglitazone, used alone or in combination, induced Akt or Erk phosphorylation in human CPCs from T2DM patients. Exposure to 0.25 mM palmitate for 16 h increased human CPC apoptosis and autophagy, evaluated by ELISA assay and LC3-II immunoblotting, respectively ($p<0.05$), in cells from both control and T2DM subjects. However, pretreatment with alogliptin, alone or in combination with pioglitazone for 1 h before exposure to palmitate, reduced palmitate-induced apoptosis and autophagy ($p<0.05$) only in human CPCs isolated from control subjects.

In conclusion, excess saturated fatty acids induce apoptosis and enhance autophagy in human CPCs. In cells from control subjects, this can be counteracted by alogliptin and pioglitazone, which activate pro-survival intracellular pathways. In contrast, CPCs from T2D patients are not protected by DPP-4 inhibitors and PPAR-gamma agonists, and this may affect the viability of the CPC compartment.

PO 70

Sitagliptin and cardiovascular outcomes during and after acute myocardial infarction: observations from TECOS

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Background: Trial results have demonstrated no effect of 3 dipeptidyl peptidase-4 inhibitors (DPP-4i) on risk for major adverse cardiovascular (CV) events. As DPP-4i reduce myocardial infarction (MI) size in animal models, we examined the effects of the DPP-4i, sitagliptin on CV outcomes during and after incident MI in the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS).

Methods: TECOS randomized 14,671 participants with type 2 diabetes (T2D) and atherosclerotic cardiovascular disease to sitagliptin or placebo, in addition to usual care. For those who had a within-trial MI, we analyzed case fatality and subsequent time to a composite outcome of cardiovascular death or hospitalization for heart failure (hHF) by treatment group, using Cox proportional hazards models left-censored at the time of the first nonfatal AMI, without and with adjustment for potential confounders in intention-to-treat analyses.

Results: During TECOS, 616 participants had at least one MI (300 allocated to sitagliptin, 316 allocated to; placebo; HR 0.95 (0.81, 1.11) of which 25 were fatal, split with 11 in the sitagliptin group and 14 in the placebo group, leaving 289 and 302 nonfatal MI respectively. Subsequently, of the 591 patients with a nonfatal MI, 87 (15%) died including 66 (11%) with CV deaths, and 57 (10%) experienced hHF. Those with MI more likely to be male (77.8% vs. 70.4%, p<0.0001); at baseline, had more coronary artery disease (89.4% vs. 73.4%, p<0.0001), prior AMI (57.7% vs. 42.0%, p<0.0001) and prior hHF (20.8% vs. 17.9%, p=0.068); were treated less commonly with metformin (75. 6% vs. 81.8%, p<0.0001)and more commonly with insulin (33.2% vs. 22.8%, p<0.0001.

The primary composite outcome occurred in 58 (20.1%; 13.9 per 100 person-years) sitagliptin group participants and in 50 (16.6%; 11.7 per 100 person-years) placebo group participants (HR1.21, 95% CI 0.83–1.77, P=0.32, adjusted HR 1.23, 95% CI 0.83–1.82, P=0.31). Similar results were seen for cardiovascular death and hHF when tested separately. On-treatment sensitivity analyses likewise revealed no significant difference in post MI outcomes between the groups.

Conclusions: In patients with T2D experiencing an MI, sitagliptin did not reduce subsequent risk of cardiovascular death or hHF, contrary to expectations deriving from pre-clinical animal model observations.

PO 71

Early initiation of sitagliptin during metformin up-titration in treatment of patients with T2DM

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Background and aims: Metformin (MET) is widely used as the first-line antihyperglycemic agent (AHA) for patients with T2DM; it is usually initiated at a low dose and up-titrated based on tolerability and glycemic response. For many patients not at glycemic goal on a sub-maximal MET dose, MET dose maximization does not result in attainment of HbA1c goal. To better understand the optimal thresholds for early addition of a second-line AHA, the safety and efficacy of MET maximization with simultaneous addition of sitagliptin (SITA) were compared to MET maximization alone in participants not at HbA1c goal on a sub-maximal dose of MET.

Materials and methods: Participants at baseline had inadequate glycemic control on no AHA or on monotherapy with MET at 1000 mg/day or on another AHA. Prior to randomization, all participants continued or transitioned to MET 1000 mg/day for a 6-10 week stabilization period, followed by a 2-week single-blind

placebo (PBO) run-in. Those with HbA1c 58 to 97 mmol/mol [7.5 to 11.0%] prior to the run-in were eligible for randomization. At randomization, participants were assigned (1:1) to SITA 100 mg/day or matching PBO. The MET dose was to be increased from 1000 mg/day to 2000 mg/day by study Week 2 and continued through Week 20. Primary objectives were to compare the effect of up-titration of MET with and without addition of SITA on reduction from baseline in HbA1c after 20 weeks of treatment and to assess the overall safety and tolerability of these regimens. Secondary objectives included the effects of treatment on the percentage of patients at the HbA1c goal of <53 mmol/mol (<7%) and reduction from baseline in fasting plasma glucose (FPG) after 20 weeks.

Results: Treatment groups were well balanced at baseline (n = 229/group, mean HbA1c = 71.1 mmol/mol [8.7%], mean FPG = 10.2 mmol/L). At Week 20, LS mean changes from baseline HbA1c were greater with SITA vs. PBO, p<0.001. At Week 20, the HbA1c goal of <53 mmol/mol (<7.0 %) was more likely to be met with SITA than with PBO overall (Relative Risk 1.7, p=0.001), and in the pre-specified subgroup with baseline HbA1c ≥69 mmol/mol (8.5 %) (Relative Risk 2.4, p=0.026). At Week 20, LS mean changes from baseline in FPG were greater with SITA vs. PBO, p=0.002. Both treatment regimens were well tolerated, with no notable between-group differences in safety or tolerability.

Conclusion: In this study of T2DM patients with inadequate glycemic control on MET 1000 mg/day, early initiation of SITA, simultaneous with MET dose maximization, was well tolerated and increased HbA1c goal attainment. These data support that initiation of SITA concomitantly with MET up-titration may be a preferred treatment-intensification strategy for many T2DM patients not at HbA1c goal on a sub-maximal dose of MET.

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