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IMPACT OF HORMONE REPLACEMENT THERAPY ON HYPERANDROGENICITY AND GLUCOSE HOMEOSTASIS IN POSTMENOPAUSAL DIABETIC WOMEN

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ABSTRACT

Hyperandrogenicity in women is closely associated with insulin resistance and a risk factor for cardiovascular disease and noninsulin-dependent diabetes mellitus. It is known that hormone replacement therapy (HRT) decreases hyperandrogenicity and improves glucose homeostasis in postmenopausal diabetic women. To investigate the role of HRT on hyperandrogenicity and glucose homeostasis in postmenopausal diabetic women. A total of 40 type 2 diabetic postmenopausal women were prospectively enrolled and followed for 12 months. The examined group consisted of 20 women who were assigned to take HRT, while the rest were left without hormone therapy. HRT consisted of 17β- estradiol (E2) 1 mg and DRSP (drospirenone) 2 mg. Fasting blood glucose (FPG), glycosylated hemoglobin (HbA1C), insulin, sex hormone binding globuline (SHBG) and total testosterone were measured, free androgen index (FAI) was calculated by formula and insulin sensitivity was determined by the homeostatic model assessment of insulin resistance (HOMA-IR). All metabolic measurements were taken at baseline and after 12 months.HRT treatment, compared with control group, was followed by a marked increase of SHBG (from 29.0 ± 12.3 to 56.0 ± 8.54 nmol/l) and significant decrease of free testosterone (5.17 ± 1.2 to 1.92 ± 1.3), FPG (7.8 ± 0.86 to 6.9 ± 0.6 mmol/l), HbA1C(7.6 ± 0.54 to 7.2 ± 0.43 % and HOMA-IR (4.23 ± 1.7 to 3.18 ± 1.4 μU/ml-mmol/l).HRT in postmenopausal diabetic women ameliorated hyperandrogenicity, accompanied by marked improvements in glucose homeostasis.

Keywords: Hormone replacement therapy, Menopause, Hyperandrogenicity, Diabetes mellitus, Insulin resistance.

INTRODUCTION

Many studies have investigated the role of estrogen during menopause; however, less attention has been paid to the role of androgens. Given the possibly opposite effects of estrogens and androgens on cardiovascular disease risk [1], it is proposed that relative androgen excess may better predict the increased risk of cardiovascular disease in women around menopause than estrogen levels alone.

Several epidemiological studies have shown that hormone replacement therapy with estrogens may prevent or reduce cardiovascular disease in postmenopausal women [2, 3] and also prevent postmenopausal bone loss [4,5]. Oral estrogens seem to favorably alter the lipoprotein profile by increasing high-density lipoprotein (HDL) cholesterol and decreasing low-density lipoprotein (LDL) cholesterol, which may be a plausible mechanism to explain at least part of the reduction of cardiovascular diseases by estrogens [6-8].

Apart from plasma lipids, hyperinsulinemia and insulin resistance also are strong and independent risk factors for cardiovascular disease [9], but the effects of estrogens on carbohydrate metabolism and insulin resistance are more controversial. However, in two large population studies, the use of conjugated estrogens recently has been reported to be associated with lower fasting insulin levels than in nonusers [10,11], and Manson [12] has shown that the use of estrogen replacement therapy was not associated with a higher incidence of noninsulin-dependent diabetes mellitus (NIDDM).

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Recent investigations have demonstrated an association between hyperandrogenicity, as indicated by low levels of sex hormone-binding globulin (SHBG), and elevated free testosterone on the one hand, and insulin resistance on the other, in both pre- and postmenopausal women [13-15]. Furthermore, a low level of SHBG is a strong and independent risk factor for development of NIDDM in women [16, 17], and postmenopausal women with manifest NIDDM seem to be relatively hyperandrogenic in comparison with healthy women of similar age and body mass index [18].

With this background, we hypothesized that hormone replacement therapy might alleviate hyperandrogenicity and insulin resistance in postmenopausal women with NIDDM. The present study was therefore performed to test this possibility.

MATERIALS AND METHODS

We prospectively studied 40 postmenopausal women with type 2 diabetes.Diabetes was diagnosed using the criteria of the World Health Organization, at least 2 years before entering the study. In order to maintain their glucose levels in an acceptable range, women with type 2 diabetes were on dietary management alone (two patients) or taking oral anti-diabetic drugs that consisted of metformin and sulfonylureas (38 patients). Each diabetic patient received a diabetic diet of 1300 kkal/day. The women were instructed not to change their diet. None of them were taking insulin. None of the women were taking anti-lipidaemic, corticosteroid or anti-convulsant therapy. The anti-diabetic medications were left unchanged during the study.

Menopause was confirmed by the absence of menstruation for at least 12 months and by high serum levels of FSH (>30 mIU/ml) and low serum levels of estradiol (E_2) (<20pg/ml).They all had intact uterus. The subjects had not received HRT previously. Gynaecological examination and mammogram were normal in all subjects.

Half of the subjects (20 women) were assigned to take HRT (DM – HRT) group. The other half (20 women) made the control group, not taking HRT (DM – non HRT group). The randomisation of the subjects has been done upon base of willingness and motivation to cooperate. Subjects in the DM - HRT group had been taking oral HRT consisting of 17β- estradiol (E2) 1 mg and DRSP (drospirenone) 2 mg - Angeliq®, Schering AG, Germany, l tablet daily for 12 months .Subjects in DM – non HRT group were followed the same way as the examined group.Exclusion criteria in both groups included *1*) hypertension (systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg); *2*) anemia; 3) various degrees of renal insufficiency;4) evidence of significant liver disease; and 5) hysterectomy or a history of recent surgery and who demonstrated significant chronic alcohol intake, were also excluded. All patients in this study gave written informed consent.

This work was approved by the local medical ethics committee and all participants gave informed consent before the onset of study.

All of the participants enrolled in our study have been contacted by telephone in three months interval in order to discover any adverse effect of HRT. All metabolic and physical examinations were performed at the onset of the study and then again after 12 months of receiving HRT. Blood blood samples were taken after a 12 h fast. HbA_1C was determined on an Cobas c 111 analyzer using commercial kits supplied from Roche Diagnostic Gmbh (Switzerland), and glucose levels were determined in an Beckman Analyzer 2 automated analyser by using commercial kits supplied from Analox Instruments Ltd, London (UK). Insulinemia, SHBG and total testosteron was determined in an Elecsys 2010 analyzer using commercial kits from Roche Diagnostics Gmbh (Switzerland).

Assessment of IR

In primary analysis, we evaluated degrees of IR by homeostatic model assessment for IR (HOMA-IR) using the formulas HOMA-IR = glucose × insulin/22.5 A higher HOMA-IR value indicates greater IR.

Assessment of hyperandrogenicity

Free testosterone presented as Free Androgen Index (FAI) .

$$FAI = 100 \times (\frac{Total\ Testosterone}{SHBG})$$

Statistical Analysis

Statistical analysis was carried out with descriptive statistics, t - test for related samples and t - test for independent samples. The data are expressed as means ± SEM. Statistical significance was set at $P < 0.05$. Data were analysedusingStatistica, version 10.0 (StatSoft) for Windows.

RESULTS

All of the women who were enrolled into the investigation completed the study. The mean age of the subjects was 49 3 ± .34 and 48.5 ± 3.1 years, and their mean body mass index (BMI) was 27 .27 ± 3.32 and 28.3 ± 2.4 kg/m^2 in the DM-HRT and DM non--HRT groups respectively. High school educated were 58,3 % and 54,2 % .

The baseline caracteristics of the subjects are given in Table 1.Two subjects in the DM-HRT group and one subject in the DM non-HRT group complained about abnormal vaginal bleeding such as metrorrhagia and four patients reported breast tenderness in the DM-HRT group. Other adverse effects were not seen.

Sex hormones

SHBG increased from 29.0 ± 12.3 nmol/L at baseline to 56.0 ± 8.54 nmol/L after HRT substitution ($P < 0.001$), and free testosterone decreased from 5.17 ± 1.2 at baseline to 1.92 ±1.3 after HRT treatment ($P < 0.001$). In control group, there was no significance observed at baseline 6.03 ± 1.1 and after 12 months of follow up 6.10 ± 0.9.

Glucose metabolism

During treatment with HRT, fasting blood glucose decreased from 7.8 ± 0.86 mmol/L at baseline to 6.9 ± 0.6 mmol/L ($P < 0.001$) after treatment. HbA1c was reduced from 7.6 ± 0.54 % before HRT substitution to 7.2± 0.43 % after substitution ($P < 0.001$). Insulinemia decreased from 12.2 ± 3.41 (µU/ml) before HRT treatment to 10.4 ± 2.92 (µU/ml) after 12 monhs of treatment with HRT (p<0.001). Regarding insulin resistance, HOMA-IR was reduced from 4.23 ± 1.7 (µU/ml-mmol/l) at baseline to 3.18 ± 1.4 (µU/ml-mmol/l) at the end of the study.

Regarding changes in the same metabolic parameters in the control grup, FPG decreased from 8.0 ± 0.9 mmol/l at the baseline to 7.8 ± 1.1 mmol/l after 12 months follow up (p=0.66), HbA1C was reduced from 7.9 ± 0.5 % at baseline to 7,7 ± 0.4 % at the end (p=0.477). Levels of insulinemia increased not significantly from 12.3 ± 3.2 µU/ml at baseline to 13.1 ± 3.7 µU/ml after 12 months (p=0.08) and HOMA-IR revealed not significant increase from the baseline 4.31 ± 1.8 µU/ml-mmol/l to 4.54 ± 1.7 µU/ml-mmol/l after 12 months (p=0.69).

Table 1. Baseline charasteristcics of postmenopausal women

	Women on HRT (*n* = 20)	**Women not on HRT (*n* = 20)**	**P**
Age (years)	49 ± 3.34	48.5 ± 3.1	N.S
BMI (kg/m^2)	27 ± 3.32	28.3 ± 2.4	N.S
FPG (mmol/l)	7.8 ± 0.86	8.0 ± 0.9	N.S
HbA1C (%)	7.6 ± 0.54	7.9 ± 0.5	N.S
Insulinemia(µU/ml)	12.2 ± 3.41	12.3 ± 3.2	N.S
HOMA-IR (µU/ml-mmol/l)	4.23 ± 1.7	4.31 ± 1.8	N.S
SHBG (nmol/l)	29.0 ± 12.3	28.0 ± 10.9	N.S
Total testosterone (nmol/l)	1.56 ± 0.45	1.69 ± 0.14	N/S
FAI	5.17 ± 1.2	6.03 ± 1.1	N.S

N/S statistically not significant for group comparison at baseline Changes in sex hormones and glucose metabolism are shown in Table 2

Table 2. Effects on HRT on Fasting Plasma Glucose (FPG), HbA1C , Insulinemia, HOMA – IR, Testosteron, SHBG & FAI

	Women on HRT (*n* = 20)	**P value**	**Women not on HRT (*n* = 20)**	**P value**	**P* value**
FPG (mmol/l) Baseline 12 months	 7.8 ± 0.8 6.9 ± 0.6	p< 0.001	 8.0 ± 0.9 7.8 ± 1.1	P=0.66	p* < 0.0001
HbA1C % Baseline 12 months	 7.6 ± 0.54 7.2 ± 0.43	p<0.001	 7.9 ± 0.5 7,7 ± 0.4	p=0.477	p* < 0.0001
Insulinemia (µU/ml) Baseline 12 months	 12.2±3.41 10.4±2.92	p<0.001	 12.3 ± 3.2 13.1± 3.7	p= 0.08	p* <0.0001
HOMA – IR (µU/ml-mmol/l) Baseline 12 months	 4.23 ± 1.7 3.18 ± 1.4	p<0.001	 4.31 ± 1.8 4.54 ± 1.7	p=0.69	P* <0.0001
SHBG (nmol/l) Baseline 12 months	 29.0±12.3 56.0±8.54	p< 0.001	 28.0±10.9 29.0±12.3	p=1.08	p* <0.0001
Tot.Testosterone (nmol/l)					

Baseline 12 months	1.56±0.45 1.08± 35	p<0.001	1.69±0.14 1.77± .13	p=0.62	p* <0.0001
FAI Baseline 12 months	 5.17 ± 1.2 1.92 ±1.3	p<0.001	 6.03 ± 1.1 6.10 ± 0.9	p=1.10	p* <0.0001

p< 0,05 statistically significant for all postmenopausal women included in adequate group at baseline and after 12 months treatment**,** p* < 0.05 statistically significant for group comparison at 12 months

DISCUSSION

In previous studies, the coupling between hyperandrogenicity and insulin resistance does not seem to have been considered. Several epidemiological cross-sectional studies have demonstrated this relationship in both pre- and postmenopausal, nondiabetic and diabetic women [13-19]. Hyperandrogenicity, as indicated by low SHBG values, also is a powerful independent risk factor for the development of NIDDM, hypertension [20], and cardiovascular disease and overall mortality [21].

Furthermore, a low SHBG level is associated closely with visceral obesity. Women with central body fat distribution have low levels of SHBG and increased free testosterone in parallel with insulin resistance [22]. Visceral obesity is a risk factor for cardiovascular disease, stroke, and NIDDM [23,24]. Low SHBG concentrations and visceral obesity may have additive effects on insulin resistance and risk to develop NIDDM, hypertension, and cardiovascular disease.

The direction of causality between hyperandrogenism and insulin resistance in women is not fully clarified. Both types of causal associations have been postulated. Studies of anabolic steroids [25, 26], PCO women [27,28], androgen treatment of female to male transsexuals [29], oral contraceptive administration [30], and studies of the effect of testosterone on insulin sensitivity in female rats [31] suggest that increased androgenicity in women may cause insulin resistance.

On the other hand, there are several pieces of evidence that insulin resistance, or rather hyperinsulinemia, may lead to hyperandrogenism. It has been shown that hyperinsulinemia increases androgen output from the ovary [32-34] and may suppress SHBG production in the liver, shown in *vitro* [35] and indirectly in clinical studies [36,37].

Furthermore, several previous studies in women with hyperandrogenism have shown that suppression of androgens into normal levels did not result in improvements in insulin resistance [38-40]. However, Moghetti [41] and Shoupe [42] have demonstrated thatantiandrogen treatment resulted in partially reversed insulin resistance.

Previously it has been suggested that insulin sensitivity is preserved in the liver but reduced in the periphery in hyperandrogenic women or female-to-male transsexuals treated with androgens [29, 43]. The diminished peripheral insulin sensitivity may be mediated via a direct effect of androgens on skeletal muscle [44-46].

In addition to alleviating androgen effects on muscle, estrogens alone also may have direct effects on skeletal muscle. Estrogens regulate insulin-induced glucose transport [47] via translocation of glucose transporter 4 [48].

Another possible mechanism for the improvement of glucose homeostasis may be an increased estrogen mediated basal and insulin-mediated suppression of hepatic glucose production because patients with NIDDM have both hepatic and extrahepatic insulin resistance [49]. Estradiol has been reported to depress hepatic glucose output [50]. In support of this possibility, Brussaard*et al* [51] had shown that estradiol treatment of postmenopausal women with NIDDM was followed by increased suppression of hepatic glucose production and improvement in HbA1c and HDL cholesterol concentrations .

The potential cause-effect sequence, implying that hyperandrogenicity induces insulin resistance and worsening of glucose metabolism, seems to be in agreement with the results reported in our study because elevated SHBG levels induced by HRT administration alleviating hyperandrogenicity was followed by an improved glucose metabolism.

However, the direction of casuality and the relationship between hyperandrogenicity and insulin resistance remain not fully clarified and need more studies and more work up on this topic.

In conclusion we may say that the main findings in the present report was an alleviation of hyperandrogenicity followed by improvement of glucose metabolism in postmenopausal women with NIDDM after hormone replacement therapy.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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