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Does androgen excess contribute to the cardiovascular risk profile in postmenopausal women with type 2 diabetes?

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Abstract

The purpose of this study was to determine if postmenopausal women with type 2 diabetes have clinical and biochemical evidence of androgen excess as a potential contributor to an increase in risk for coronary heart disease when compared with women without diabetes. Fasting glucose, insulin, lipids, sex hormone–binding globulin (SHBG), and sex steroids (from pooled samples) (total testosterone and free testosterone [non–SHBG-T], androstenedione [A-dione], total estrogens) were measured at baseline in 16 postmenopausal women with type 2 diabetes treated with diet or a sulfonylurea and 17 age-matched controls. Measurements of glucose, insulin, and sex steroids were repeated at hourly intervals for 3 hours after oral glucose administration. Hirsutism scores and insulin sensitivity (homeostasis model assessment [HOMA] insulin sensitivity [SI]) were obtained. Women with type 2 diabetes were more hyperglycemic, hyperinsulinemic, and insulin-resistant (HOMA SI, 46.7 ± 7.0 vs 12.9 ± 2.0, $P < .001$), and had higher total to high-density lipoprotein cholesterol (TC/HDL) ratios, lower SHBG (20.8 ± 3.5 vs 59.3 ± 14.4 nmol/L, $P < .05$), higher non–SHBG-T (0.225 ± 0.025 vs 0.135 ± 0.021 nmol/L, $P < .05$), and higher hirsutism scores (1.1 ± 0.3 vs 0.3 ± 0.2, $P = .004$) than those without diabetes. No changes in sex steroids occurred after the oral glucose challenge. HOMA SI and area under the curve for glucose correlated significantly with SHBG ($r = -0.42$), non–SHBG-T ($r = 0.40$), and TC/HDL ($r = 0.41$) (all $P < .05$) in the combined groups. Postmenopausal women with type 2 diabetes have both clinical and biochemical evidence of androgen excess that may contribute to more adverse cardiovascular risk profiles.

1. Introduction

Type 2 diabetes has a stronger negative impact on risk for both the occurrence of and the mortality from coronary heart disease (CHD) in women than men [1-3]. This sex-specific acceleration in CHD risk is partially explained by increases in the severity of conventional risk factors, including obesity, hypertension, and dyslipidemia; however, the reasons underlying the greater prevalence of these risk factors remain unexplained [2,4].

This increase in conventional risk factors together with the observation that an accelerated risk for CHD precedes the onset of clinical hyperglycemia suggests the presence of sex-specific factors that contribute to the overall risk profile [5]. Hyperinsulinemia and insulin resistance precede the onset of clinical diabetes by 10 to 20 years [6-9]. In women with polycystic ovary syndrome (PCOS), hyperinsulinemia contributes to the overproduction of ovarian androgens, which in turn contribute to a greater prevalence of CHD risk factors in this group of women [10]. It is possible that prolonged periods of hyperinsulinemia preceding the onset of type 2 diabetes in women may alter ovarian steroidogenesis toward androgen overproduction with resulting alterations in circulating lipids and an augmentation in CHD risk.

The present study was designed to investigate the hypothesis that women with type 2 diabetes have insulin-mediated androgen excess that contributes to CHD

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Table 1
Clinical characteristics of subjects

	Type 2 diabetes (n = 16)	Controls (n = 17)	*P*
Age (y)	59.1 ± 1.3	56.9 ± 1.1 NS	NS
Race	13 W/3 B	17 W	
BMI (kg/m^2)	33.9 ± 2.1	29.4 ± 1.7	.04
WHR	0.85 ± 0.01	0.81 ± 0.01	.04
HbA_{1c} (%)	7.7 ± 0.4	5.7 ± 0.1	.001
Age at menopause	46.9 ± 3.3	49.9 ± 1.1	NS
Years postmenopausal	8.9 ± 1.7	7.4 ± 0.9	NS
SBP (mm Hg)	137 ± 7	125 ± 5	NS
DBP (mm Hg)	83 ± 3	78 ± 2	NS
MAP (mm Hg)	101 ± 4	94 ± 2	NS
Pregnancies (n)	3.8 ± 0.7	3.6 ± 0.8	NS
LH (mIU/mL)	25.5 ± 3.7	30.9 ± 3.8	NS
FSH (mIU/mL)	57.3 ± 5.4	69.2 ± 5.4	NS
Hirsutism score	1.1 ± 0.3	0.3 ± 0.2	.004
Urine albumin (mg/g creatinine)	54.4 ± 22.1	6.1 ± 1.3	.004
TC (mmol/L)	5.8 ± 0.3	5.2 ± 0.2	NS
TGs (mmol/L)	2.2 ± 0.3	1.4 ± 0.2	NS
Total HDL (mmol/L)	1.01 ± 0.04	1.37 ± 0.07	NS
LDL (mmol/L)	3.7 ± 0.3	3.2 ± 0.2	NS
TC/HDL	5.4 ± 0.3	4.0 ± 0.3	<.05
Lp(a)	19.9 ± 5.0	26.7 ± 6.2	NS

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

risk profiles, in part, through alterations in circulating lipid profiles.

2. Materials and methods

2.1. Patients

The study was approved by the Institutional Review Board of the University of Pittsburgh. All patients gave informed written consent before study participation. Participants included 16 postmenopausal women with type 2 diabetes treated with diet alone (n = 8) or a sulfonylurea (n = 8) and 17 postmenopausal women without diabetes by oral glucose tolerance testing or a known family history of type 2 diabetes. Exclusion criteria included a history or bilateral oophorectomy, a hirsutism score of more than 6, a history of oligoamenorrhea, or unexplained infertility in an effort to exclude women with PCOS. The groups were matched for age and years postmenopause (Table 1). All participants described sedentary lifestyles. No subjects were taking metformin, glitazones, acarbose, insulin, lipid-lowering therapy, or medications known to interfere with glucose metabolism or insulin sensitivity (ie, glucocorticoids, β-blockers, or thiazide diuretics). Women on hormone replacement therapy discontinued this for 3 months before the study.

2.2. Screening procedures

Physical examination included measurement of seated blood pressure, height, weight, waist-to-hip ratio (WHR), and a modified hirsutism scores [11]. Screening laboratory testing included measurement of a thyroid-stimulating hormone, hematocrit, serum creatinine, and gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]). Menopausal status was established by the absence of menses for 1 year.

2.3. Protocol

Subjects were instructed to follow a 150-g carbohydrate diet for 2 days and a 10-hour overnight fast before admission to the General Clinical Research Center at the University of Pittsburgh Medical Center at 7:30 AM on the study day. Sulfonylurea-treated subjects were asked to withhold their medication for 2 days before the study day. An antecubital intravenous catheter was placed and maintained with a slow infusion of 0.9% saline before the initiation of blood sampling at 8:00 AM for metabolic profiles (glucose, insulin, C-peptide); lipids (total cholesterol [TC], triglycerides [TGs], high-density lipoprotein cholesterol [HDL-C], lipoprotein (a) [Lp(a)]); sex hormones (total testosterone [TT], total estrogens, androstenedione [A-dione], DHEAS); and sex hormone–binding globulin (SHBG). Sampling for metabolic profiles and sex hormones was repeated at 8:30 and 9:00 AM, followed by oral administration of 100 g of dextrose. Repeat sampling for metabolic profiles was performed at 30, 60, 90, 120, and 180 minutes. Repeated sampling for sex hormones was performed at hourly intervals. At the conclusion of the study, all subjects were fed and discharged. Sulfonylurea-treated subjects resumed their medication before the meal.

2.4. Analytical methods

Plasma glucose concentrations were determined using the glucose oxidase method with a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin, C-peptide, cholesterol (TC, low-density lipoprotein [LDL-C], HDL-C), and TGs were measured using previously described methods [12,13]. Serum Lp(a) was quantitatively determined using an enzyme-linked immmunosorbent assay technique. Measures of insulin resistance were obtained using homeostasis model assessment (HOMA) [14]. Sex hormone–binding globulin was measured by IRMA (Diagnostic Systems Laboratories, Webster, TX). Basal levels of TT, A-dione, DHEAS (Coat-A-Count solid-phase radioimmunoassay, Diagnostic Products, Los Angeles, CA), and total estrogens (ICN Pharmaceuticals, Costa Mesa, CA) were determined from pooled basal samples. The radioimmunoassay for total estrogens has 100% cross-reactivity with both 17β-estradiol and estrone and 9% or less cross-reactivity with estriol, 17a-estradiol, and equilin.

The determination of non–SHBG-T was based on the separation, by 500 g/L ammonium sulfate precipitation, of serum SHBG-bound testosterone after incubation with ^{3}H-testosterone at 23°C [15]. The non–SHBG-T concentration was calculated by multiplying the percentage of tracer in the supernatant (not bound to SHBG) by the TT concentration. Postglucose samples were based on a single

Table 2
Glucose, insulin, C-peptide, and androgens levels at baseline (0) and at 1, 2 and 3 hours after glucose administration

	Time (h)			
	0	1	2	3
Glucose (mmol/L)				
DM	9.5 ± 0.6**	16.1 ± 0.8**	17.7 ± 0.8**	16.9 ± 1.1**
Controls	5.3 ± 0.1	9.2 ± 0.6	7.6 ± 0.4	5.9 ± 0.4
Insulin (pmol/L)				
DM	113 ± 16**	310 ± 51**	448 ± 86**	436 ± 84**
Controls	54.3 ± 8	606 ± 74	486 ± 67	264 ± 37
C-peptide (nmol/L)				
DM	1.9 ± 0.3**	2.4 ± 0.3	3.0 ± 0.4	3.1 ± 0.5*
Controls	0.7 ± 0.1	2.1 ± 0.3	2.3 ± 0.4	1.9 ± 0.2
TT (nmol/L)				
DM	0.93 ± 0.11	0.85 ± 0.10	0.75 ± 0.09	0.76 ± 0.09
Controls	0.79 ± 0.10	0.71 ± 0.71	0.67 ± 0.07	0.65 ± 0.06
A-dione (nmol/L)				
DM	20.8 ± 4.3	22.6 ± 2.9	21.4 ± 2.5	20.6 ± 2.4
Controls	21.5 ± 5.4	23.4 ± 3.6	27.8 ± 4.5	30.1 ± 6.1
DHEAS (nmol/L)				
DM	2.2 ± 0.3	2.2 ± 0.4	2.1 ± 0.3	2.1 ± 0.3
Controls	3.6 ± 0.7	3.3 ± 0.5	3.1 ± 0.5	3.3 ± 0.6

* $P < .5$ vs controls.
** $P < .01$ vs controls.

plasma determination. The area under the curve (AUC) for glucose, insulin, and C-peptide responses was determined as the mean incremental response above baseline [13]. Intra- and interassay variability for all assays ranged from 1.5% to 7.4% and 4.1% to 6.5%, respectively.

2.5. Statistics

Comparisons of clinical characteristics of diabetic patients and controls were carried out using Student *t* test. Where necessary, variables were transformed to meet the *t* test's normality assumption. Comparisons of metabolic variables were carried out using *t* tests, and for adjustment for body mass index (BMI), regression analysis. Differences in temporal patterns of sex hormones before and after glucose administration were assessed using repeated-measures analysis of variance. Correlations among pairs of study variables were examined using Spearman correlation coefficient because the relationships were not generally linear. Partial correlations adjusting for BMI were calculated using Spearman coefficient after rank-transforming the variables.

3. Results

3.1. Clinical characteristics of subjects

The average duration of type 2 diabetes was 6.2 ± 1.8 years. Women with type 2 diabetes had significantly higher BMI, WHR, hirsutism scores, HbA_{1c}, and urine microalbumin concentrations than those without diabetes ($P < .05$). LH and FSH levels were slightly but not significantly lower in women with diabetes. No differences in parity or blood pressure were observed (Table 1).

3.2. Metabolic measures

Glucose, insulin, and C-peptide levels at baseline and at hourly intervals after the ingestion of glucose are summarized in Table 2. Fasting plasma glucose, insulin, and C-peptide were significantly higher in diabetic subjects ($P < .05$). After the ingestion of oral glucose, incremental $AUC_{glucose}$ (6.3 ± 0.4 vs 2.5 ± 0.2 mmol/L, $P < .05$) was higher in women with diabetes, whereas $AUC_{insulin}$ (242 ± 57 vs 407 ± 39 pmol/L, $P < .05$) and $AUC_{C\text{-}peptide}$ (0.8 ± 0.1 vs 1.2 ± 0.2, $P < .05$) responses were higher in those without diabetes. Women with diabetes were more insulin-resistant than controls (HOMA insulin sensitivity [SI], 46.7 ± 7.0 vs 12.9 ± 2.0, $P < .001$). The TC/HDL-C was higher in women with diabetes (Table 1). No significant differences were observed between the 2 groups for TC, LDL-C, Lp(a), TG, or HDL-C.

Sex hormone–binding globulin was significantly lower in the women with diabetes (Fig. 1A), whereas non–SHBG-T (Fig. 1B) and percentage of free T (2.1% ± 0.1% vs 1.6% ± 0.2%, $P = .02$) were significantly higher both before and after adjusting for between-group differences in BMI. No between-group differences for basal measures of TT (0.93 ± 0.11 vs 0.79 ± 0.10 nmol/L), A-dione (20.8 ± 4.3 vs 21.5 ± 5.4 nmol/L), DHEAS (2.2 ± 0.3 vs 3.6 ± 0.7 nmol/L), or total estrogens (60 ± 6 vs 54 ± 7 pg/mL) were observed. There were no significant changes in any of these hormonal levels in either group in response to the oral glucose challenge (Table 2).

3.3. Correlations

Correlations with metabolic measures were sought to begin to explain the higher non–SHBG T levels in women with diabetes. When both groups were combined, significant correlations were observed for HOMA SI with SHBG ($r = -0.42$, $P = .015$), non–SHBG-T ($r = 0.41$, $P = .020$), and the TC/HDL ratio ($r = 0.40$, $P = .02$). $AUC_{glucose}$ was also significantly correlated with each of these measures ($r = -0.61$, $P = .006$; $r = 0.38$, $P = .047$; $r = 0.48$, $P = .01$, respectively). These correlations were not observed in each group individually. Correlations were not observed for LH, WHR, or basal and glucose-stimulated insulin concentrations with any measure of testosterone, A-dione, DHEAS, or lipids in either the combined or individual groups.

3.4. Treatment effects

Among subjects with type 2 diabetes, those treated with diet alone (n = 8) had a shorter disease duration (3.1 ± 1.5 vs 8.6 ± 2.8 years, $P = .04$) and a lower HbA_{1c} level (6.9% ± 0.6% vs 8.3% ± 0.5%, $P = .04$) than those treated with a sulfonylurea (n = 8). Despite these differences, no

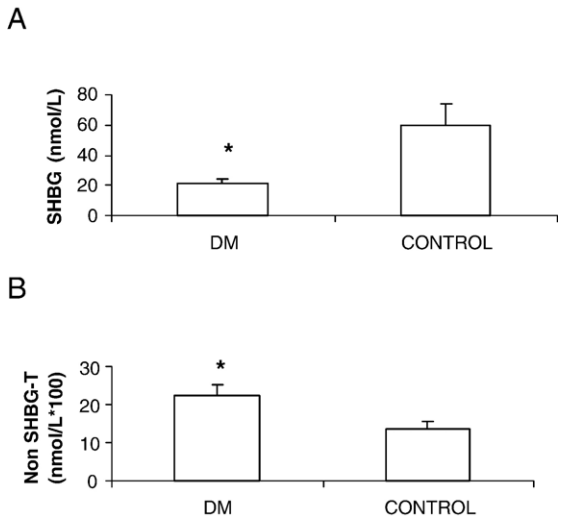

Fig. 1. SHBG (nmol/L) (A) and non–SHBG-T (nmol/L × 100) (B) in women with type 2 diabetes and controls. Sex hormone–binding globulin levels were significantly lower in women with diabetes before (P = .006) and after (P = .045) adjusting for differences in BMI between the 2 groups. Significant differences for non–SHBG-T were present before (P = .0032) and after (P = .02) adjusting for BMI.

differences were observed in basal and glucose-stimulated glucose, insulin, and C-peptide responses during the oral glucose tolerance testing, fasting lipids, SHBG, or sex steroids (data not shown).

4. Discussion

The present study demonstrates that postmenopausal women with well-controlled type 2 diabetes are insulin-resistant with evidence of both biochemical and clinical androgen excess when compared with healthy nondiabetic women with no known risk factors for diabetes other than obesity (Table 1). The observed associations in the combined groups between measures of non–SHBG-T and the TC/HDL ratio, a marker of dyslipidemia, suggest that androgen excess may contribute to the burden of CHD risk factors observed in women with and without type 2 diabetes. These findings are particularly striking in that an effort was made to exclude women with PCOS, a frequently undiagnosed condition associated with hyperandrogenism and increased risk for impaired glucose tolerance and type 2 diabetes [16,17].

Although elevations in non–SHBG-T, a measure of free or biologically active testosterone, have been reported in previous studies of women with type 2 diabetes [18-20], to our knowledge, this is the first study to demonstrate a correlation between insulin sensitivity and biochemical measures of androgen excess in postmenopausal women with type 2 diabetes and clearly defined levels of glycemic control as measured by HbA_{1c}. In one study of postmenopausal Hispanic women with type 2 diabetes, higher free testosterone, estradiol, and estrone levels were reported [20]. Differences in TT and SHBG approached significance (ie, $P > .05$ and $P < .06$) only when statistical outliers were removed, making it unclear how many women were actually evaluated and whether the outliers may have represented women with preexisting PCOS. Similar to our findings, no differences were observed between diabetic women treated with diet or sulfonylureas. Andersson et al [19] reported lower SHBG and a higher free T index (TT/SHBG) as well as higher estrone levels from a single morning blood sample in postmenopausal women with type 2 diabetes relative to controls. These previous studies did not evaluate current level of glycemic control or screen for a history of PCOS among participants, which may have affected their results. We did not observe any differences in baseline total estrogens in this study. This may be explained by the fact that specific assays for estrone and estradiol were not performed as was reported in these prior studies [19,20].

Several potential explanations exist for the elevations in androgen levels in postmenopausal diabetic women reported in this study as well as others [17-19]. Sex hormone–binding globulin levels are reduced in the presence of obesity, PCOS, hyperinsulinemia, and diabetes [21-23]. Women with diabetes in this study had a higher BMI, WHR (Table 1), and basal insulin levels than controls, all of which are likely to have contributed to the low SHBG concentrations. It is possible that the reduction in SHBG permits more testosterone to circulate in its unbound or active form. However, if low SHBG alone accounted for the elevations in non–SHBG, equilibrium would be anticipated with an eventual reduction in TT concentrations. Instead, comparable levels of total testosterone were observed in each group suggesting either an increase in androgen production or a decrease in testosterone clearance in women with diabetes.

It is tempting to implicate the ovary as the source of androgen excess in postmenopausal women with diabetes [10,18,24]. The ovary of postmenopausal women remains hormonally active, with an increase in the ratio of androgen to estrogen production [19,25]. The observed increases in non–SHBG-T and the reduction in SHBG are similar to what is reported in premenopausal and perimenopausal women with PCOS [15,25], where androgen overproduction is mediated in part by high circulating levels of LH combined with elevated insulin concentrations [26]. Elevations in LH in postmenopausal women have been reported to contribute to stromal hyperplasia and an increase in ovarian testosterone production [27]. Although LH levels were similar in women with and without diabetes (Table 1) in this study, it is possible that preceding hyperinsulinemia may have interacted with elevations in LH to increase the activity of ovarian cytochrome P450c17α with subsequent elevations in basal and gonadotrophin-releasing hormone–stimulated 17-hydroxyprogesterone (17-OHP), the precursor for androgen production [26]. In our study, no correlations were observed between basal or AUC insulin with any androgen; however, 17-OHP levels were not measured as an index of the P450c pathway.

The adrenal is also an important source of androgen production in women [28]. Stress-induced alterations in the hypothalamic-pituitary-adrenal axis with elevations in corticotrophin-releasing factor, corticotropin, and cortisol have been observed in women with type 2 diabetes [29,30]. This could drive an increase in adrenal androgen production. However, DHEAS, a marker of adrenal androgen production, was nonsignificantly lower in women with diabetes in this as well as in other studies [31]. Measurements of basal adrenal androgens are not sufficient to eliminate the adrenal as a potential contributor or androgen excess in women with type 2 diabetes. Stimulated measures of other adrenal hormones, including 17-OHP, 17-hydroxypregnenolone, and DHEA would help to further clarify any adrenal contribution to androgen excess [32].

We hypothesize that an increase in ovarian androgen production contributes to the higher levels of free testosterone reported in women with diabetes. Similar to women with PCOS, insulin-mediated overproduction of androgens may be present early in the course of the progression from normoglycemia to hyperglycemia [10]. As beta-cell function declines, insulin resistance persists in the progression of type 2 diabetes [33]. Insulin resistance may thus serve as a marker of antecedent hyperinsulinemia that influences ovarian steroidogenesis toward androgen overproduction and thus contributes to CHD risk. This is illustrated by a recent study describing evidence of polycystic-appearing ovaries by ultrasonography, with biochemical hyperandrogenism in premenopausal women with type 2 diabetes [34].

Despite higher baseline androgens in the diabetic women, there was no incremental change in TT, A-dione, or DHEAS after the glucose challenge in either group. These findings differ from those reported in a previous study of premenopausal women with PCOS in which increases in TT and A-dione correlated with incremental insulin responses after an oral glucose challenge [35]. The fact that postmenopausal women were studied, together with the attenuated insulin response to oral glucose observed in diabetic women, may have contributed to the different findings. The absence of a rise in androgens in the control population despite a robust increase in insulin after oral glucose is consistent with patterns observed in eumenorrheic premenopausal women [35].

The findings in this study are limited by the relatively small sample size, which may have obscured the ability to detect significant differences in lipid parameters other than the TC/HDL ratio between the 2 groups. However, our findings of androgen excess are not only consistent with results from prior studies [1,19,20], but also add new information that elevations in androgens persist when PCOS is excluded by clinical criteria.

In summary, postmenopausal women with type 2 diabetes have both clinical and biochemical evidence of androgen excess. Although the pathogenesis of androgen excess in women with diabetes is not thoroughly understood, it may contribute to the greater burden of CHD risk factors described in these women.

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