Association of Hyperandrogenemia and Hyperestrogenemia With Type 2 Diabetes in Hispanic Postmenopausal Women

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OBJECTIVE — Accumulating evidence suggests that hyperandrogenemia may be a risk factor for coronary heart disease (CHD) in women. The present study was carried out to test the hypothesis that hyperandrogenemia is associated with type 2 diabetes in women and thus may contribute to the increased risk of CHD in women with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Sex hormones, sex hormone-binding globulin (SHBG), and risk factors for CHD were measured in 20 postmenopausal women with type 2 diabetes and in 29 control subjects. All of the diabetic and control subjects were Hispanic women aged ≥55 years who were not taking hormone replacement therapy, lipid-lowering drugs, or insulin and who were otherwise randomly chosen from a cohort of stroke-free subjects from the Northern Manhattan Stroke Study.

RESULTS — Mean age, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, blood pressure, and smoking were not significantly different between cases and control subjects, but waist-to-hip ratio (WHR) was significantly higher in the diabetic subjects (P = 0.01). The mean levels of free testosterone (FT) (P = 0.01), dehydroepiandrosterone sulfate (P < 0.04), and estradiol (P = 0.01) (controlled for WHR) were significantly higher in the diabetic subjects; with the statistical outliers removed, the testosterone (P = 0.05) and androstenedione (P = 0.002) levels (controlled for WHR) were also significantly higher in the diabetic subjects. The mean levels of estrone, cortisol, and SHBG were not significantly different. The results were similar in the 10 diabetic subjects treated with diet only. Significant positive correlations (controlled for age and BMI) were observed between FT or testosterone and cholesterol, LDL cholesterol, and blood pressure.

CONCLUSIONS — Postmenopausal Hispanic women with type 2 diabetes had both hyperandrogenemia and hyperestrogenemia, and testosterone or FT correlated positively with risk factors for CHD. Hyperandrogenemia may be a link between diabetes and CHD in women.

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Considerable evidence suggests a relationship between androgenticity and coronary heart disease (CHD) in women. An increase in waist-to-hip ratio (WHR), which appears to be an androgenic pattern of obesity in women (1–6), has been reported to be associated in women with CHD (1,7,8) and coronary artery disease (CAD) (9–12). Hirsutism (10) and polycystic ovary syndrome (PCOS) (13), which are androgenic states, have also been found to be associated with CAD. A positive relationship between free testosterone (FT) level and the degree of CAD in women has recently been reported (14). Support for an androgen-CAD relationship and evidence that it may be a cause-and-effect relationship has been reported in female monkeys in which testosterone administration was found to exacerbate diet-induced atherosclerosis independently of any effects on risk factors for CHD (15).

A possible mechanism for an androgen-CAD relationship is that androgenicity in women may underlie the expression of risk factors for CHD; this possibility is suggested by the positive relationship of androgens to risk factors for CHD in women (14,16). An increase in the levels of testosterone or FT with the risk factors hypertension (17,18), smoking (19), and increased WHR (3–6) and a positive correlation of testosterone or FT with blood pressure level (3,14,17,18), WHR (3,5), BMI (6,14), and insulin level (3,14,20,21) have been reported in women. Furthermore, the androgenic states PCOS (22–24) and increased WHR (3,7–9,12,25,26) have both been reported to be associated with risk factors for CHD, and androgen administration to women has been reported to selectively increase visceral adipose (27), which in turn may cause the expression of risk factors for CHD (28) and/or serve as a marker for them (14). Because type 2 diabetes is one of the strongest risk factors for CHD in women (29), the present study was carried out to determine whether hyperandrogenemia is also associated with type 2 diabetes.
diabetes in women. To this end, the serum levels of sex hormones and sex hormone-binding globulin (SHBG) were compared in 20 postmenopausal Hispanic women with type 2 diabetes and 29 healthy control subjects.

**RESEARCH DESIGN AND METHODS** — A total of 20 postmenopausal women with type 2 diabetes and 29 nondiabetic control subjects were studied. The diabetic and control subjects were women from the stroke-free control population enrolled in the Northern Manhattan Stroke Study, a prospective community-based study of stroke (30), who were randomly chosen after the following criteria were fulfilled: aged >55 years (at which age essentially all women are expected to be postmenopausal) (31); Hispanic ethnicity; not taking hormone replacement therapy, insulin, or lipid-lowering drugs; no major medical disorder other than type 2 diabetes or other risk factors for CHD; and a blood sample had been drawn within the past 2 years. Diabetes was defined as a fasting blood glucose level >127 mg/dl, oral hypoglycemic agent use, or a history of diabetes. Of the 20 subjects with diabetes, 10 were taking oral hypoglycemic agents, and 10 were treated with diet only. Of those taking oral hypoglycemic agents, all 10 were taking a sulfonylurea; in addition, 3 were taking metformin, and 1 was taking troglitazone.

Venous blood samples were drawn in the morning with the subject fasting and the serum stored airtight at −70°C for <2 years before analysis. Hormones were measured by radioimmunoassay (RIA). Materials for the RIA of testosterone (nonextraction coated-tube method), FT (nonprotein-bound testosterone), and dehydroepiandrosterone sulfate (DHEAS) were obtained from Diagnostic Products (Los Angeles, CA), and for estradiol (third generation), estrone, androstenedione, cortisol, and SHBG from Diagnostic Systems Laboratories (Webster, TX). Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels were measured using a Hitachi 705 automated spectrometer (Boehringer Mannheim, Indianapolis, IN).

Statistical analyses were performed using SPSS Version 6.1 (SPSS, Chicago, IL) on a Macintosh Performa 6300 (Apple Computer, Cupertino, CA) computer to calculate means, SEM, outliers (using box plots), and partial correlation coefficients. Each of the variables measured was found to be normally distributed when using the Kolmogorov-Smirnov test. Analysis of variance (with WHR as a covariate) was used to compare the means of the variables, and Fisher's exact test was used to compare the number of smokers between the control and diabetic groups. The significance of the difference between the slopes of two regression lines was determined by comparing the means of the squared Studentized residuals.

**RESULTS** — A comparison of the non-hormonal variables determined in the diabetic and control subjects is shown in Table 1. Mean age, BMI, and other risk factors for CHD were not significantly different between these two groups. The mean WHR was significantly higher (P = 0.02) in the diabetic subjects; thus, the comparisons were controlled for WHR. Notwithstanding the decreased statistical power resulting from the smaller number of diabetic subjects, this group of diabetic subjects still showed hyperandrogenemia and hyperestrogenemia. Thus, these findings could not be attributed to an effect of the oral hypoglycemic agents.

To determine whether FT or testosterone correlated significantly with risk factors for CHD other than diabetes, correlations (controlled for age and BMI) were carried out in the diabetic and control subjects separately. In the diabetic subjects, FT correlated with diastolic blood pressure (r = 0.48, P = 0.04). In the control subjects, testosterone correlated with systolic blood pressure (r = 0.50, P < 0.01), total cholesterol (r = 0.41, P < 0.04), and LDL cholesterol (r = 0.38, P = 0.05), whereas FT correlated with systolic blood pressure (r = 0.49, P < 0.01) and total cholesterol (r = 0.41, P < 0.04). SHBG correlated inversely with FT/testosterone in both the diabetic (r = −0.72, P < 0.001) and control subjects (r = −0.46, P < 0.02); the slopes of the regression lines showed no significant difference between these two groups (P = 0.95).

**CONCLUSIONS** — In the present study, the mean levels of the androgens FT and DHEAS were significantly higher in the diabetic subjects than in the control subjects. When the statistical outliers were

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**Table 1— Comparison of variables between diabetic and control subjects**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Diabetic subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.8 ± 0.8</td>
<td>65.6 ± 0.9</td>
<td>0.345</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 ± 0.81</td>
<td>28.9 ± 0.86</td>
<td>0.980</td>
</tr>
<tr>
<td>WHR</td>
<td>0.858 ± 0.017</td>
<td>0.929 ± 0.022</td>
<td>0.013</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>147 ± 3.4</td>
<td>154 ± 4.7</td>
<td>0.211</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85.4 ± 2.0</td>
<td>90.5 ± 2.8</td>
<td>0.137</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>206 ± 6.1</td>
<td>201 ± 9.7</td>
<td>0.702</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>131.6 ± 5.9</td>
<td>126.7 ± 8.8</td>
<td>0.631</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>50.8 ± 2.68</td>
<td>47.9 ± 3.24</td>
<td>0.495</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>116 ± 11.7</td>
<td>134 ± 15.5</td>
<td>0.349</td>
</tr>
<tr>
<td>Current cigarette smokers</td>
<td>5 (17)</td>
<td>1 (5)</td>
<td>0.379</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>—</td>
<td>6.6 ± 1.3</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are n, means ± SEM, or n (%).
Hyperandrogenemia in type 2 diabetes

Table 2—Comparison of hormone and SHBG levels between diabetic and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Diabetic subjects</th>
<th>P*</th>
<th>P without outliers*</th>
<th>Diabetic subjects treated with diet only</th>
<th>P*</th>
<th>P without outliers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone (pg/ml)</td>
<td>19.6 ± 1.5</td>
<td>25.5 ± 3.1</td>
<td>0.078</td>
<td>0.267</td>
<td>28.5 ± 5.6</td>
<td>0.041</td>
<td>0.398</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>22.0 ± 1.2</td>
<td>27.5 ± 1.7</td>
<td>0.013</td>
<td>0.003</td>
<td>30.8 ± 2.7</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.27 ± 0.04</td>
<td>0.29 ± 0.02</td>
<td>0.483</td>
<td>0.053</td>
<td>0.30 ± 0.03</td>
<td>0.543</td>
<td>0.113</td>
</tr>
<tr>
<td>FT (pg/ml)</td>
<td>0.65 ± 0.10</td>
<td>1.00 ± 0.10</td>
<td>0.011</td>
<td>0.003</td>
<td>1.07 ± 0.17</td>
<td>0.022</td>
<td>0.009</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>138 ± 17.4</td>
<td>97 ± 19.5</td>
<td>0.375</td>
<td>0.055</td>
<td>96 ± 32.6</td>
<td>0.557</td>
<td>0.009</td>
</tr>
<tr>
<td>DHEAS (µg/dl)</td>
<td>55.6 ± 4.3</td>
<td>81.0 ± 9.2</td>
<td>0.037</td>
<td>0.037</td>
<td>76.5 ± 10.1</td>
<td>0.162</td>
<td>0.162</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>1.30 ± 0.12</td>
<td>1.58 ± 0.13</td>
<td>0.083</td>
<td>0.002</td>
<td>1.57 ± 0.13</td>
<td>0.146</td>
<td>0.003</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>11.7 ± 1.0</td>
<td>12.0 ± 1.6</td>
<td>0.860</td>
<td>0.862</td>
<td>10.9 ± 1.2</td>
<td>0.458</td>
<td>0.593</td>
</tr>
</tbody>
</table>

Data are n or means ± SEM. *Controlled for WHR.

excluded, the mean levels of androstenedione and total testosterone also were significantly higher. Thus, the diabetic subjects exhibited hyperandrogenemia with elevation of the main androgen, testosterone, and two weaker androgens (DHEAS and androstenedione). All of the comparisons were controlled for WHR, the mean value of which was significantly higher in the diabetic subjects. An increase in the levels of dehydroepiandrosterone (DHEA) (32), DHEAS (33), and androstenedione (32) has been reported previously in women with type 2 diabetes, while one study reported a decrease in DHEA (5), and another reported no significant difference in testosterone or FT (34). Although >90% of DHEAS and ~50% of androstenedione and testosterone derive from the adrenal in women (35), the cortisol level was found to be normal in the diabetic subjects in the present study. An increase in cortisol, DHEA, and DHEAS levels has been reported in postmenopausal women with PCOS, obesity and diabetes compared with women with PCOS and obesity without diabetes (36).

That FT was more strongly related to diabetes than testosterone in the present study implicates SHBG, whose concentration appears to affect the ratio of FT to testosterone (37). Indeed, the ratio of FT to testosterone correlated inversely in both the diabetic and control subjects. But the mean SHBG level was only marginally (P = 0.055) decreased in the diabetic subjects and only after removing the statistical outliers. Two previous studies, however, have reported a decreased SHBG level and an increased testosterone/SHBG ratio (which was used as an index of FT level) in postmenopausal women with type 2 diabetes (5) or impaired glucose tolerance (38). The present study appears to be the first to report an increase in directly measured FT in postmenopausal women with type 2 diabetes. A low SHBG level has also been reported to be an independent risk factor for type 2 diabetes in women (39,40). The SHBG level has been reported to be elevated in postmenopausal women with insulin-treated diabetes (41).

The mean estradiol level was also found to be significantly increased in the diabetic subjects in the present study. An increase in the estradiol and estrone levels has been reported previously in postmenopausal women with insulin-treated diabetes (41), and an increase in the estrone level but not the estradiol level has been reported in postmenopausal women with non-insulin-treated diabetes (5). The main source of estrogen in postmenopausal women appears to be the aromatization of plasma androstenedione to estrone (42). Estradiol is produced both by the interconversion of estrone (43) and the aromatization of testosterone (44). Although this peripheral aromatization of androgens to estrogens has been reported to correlate positively with adiposity (45), the BMI of the diabetic subjects in the present study did not differ significantly from that of the control subjects. Although the WHR of the diabetic subjects was significantly greater than that of the control subjects, the hyperandrogenemia was observed after statistically controlling for WHR. Another possible mechanism for the hyperandrogenemia is that the increased levels of androstenedione and testosterone in the diabetic subjects may lead through mass action to an increase in estrogen product. The hyperandrogenemia that occurs in patients with other disorders, such as PCOS (46,47) and hirsutism (6), however, has been reported to be associated with normal estrogen levels. The concurrence of hyperandrogenemia and hyperestrogenemia has been reported in women with increased WHR (4), hypertension (17), and refractory depression (48). That hyperandrogenemia and hyperestrogenemia were also observed in the diabetic subjects treated with diet only indicates that these abnormalities were not effects of the oral hypoglycemic agents.

The finding of hyperandrogenemia in the diabetic subjects in the present study is consistent with the hypothesis that hyperandrogenemia may underlie and link risk factors for CHD in women (14,16). But whether the hyperandrogenemia and diabetes are causally related and, if so, which is the cause are unclear. The association between hyperandrogenemia and diabetes in premenopausal women with PCOS (20,21), together with a positive correlation between testosterone and insulin (3,20,21) in these women, supports the possibility of a cause-and-effect relationship. The positive correlation of testosterone with insulin (5,14) and glucose (49) in postmenopausal women provides further support. That testosterone may underlie diabetes by producing insulin resistance is suggested by reports that administration of testosterone esters (50) or methyltestosterone (51) to healthy premenopausal women induced insulin resistance. Furthermore, it has been reported that lowering the androgen level in hyperandrogenemic women with each of three different drugs resulted in a decrease in insulin resistance (52). Thus, these observations suggest that, in women, hyperandrogenemia may lead to insulin resistance and hyperinsulinemia.
There is also evidence, however, that hyperinsulinemia could give rise to hyperandrogenemia. Marked hyperinsulinemia secondary to severe insulin resistance of different etiologies appears to be associated with hyperandrogenemia (53). Also, a depressing effect of insulin on the SHBG level (54) might increase the FT/testosterone ratio (37) and thus the androgenicity inasmuch as FT may be the biologically active component of total testosterone. Decreasing both the insulin level (55) and insulin resistance (47,56,57) in women with PCOS by using drugs has been reported to decrease testosterone (55–57), FT (56,57), hyperandrogenemia. However, insulin infusion in premenopausal women, either healthy or with PCOS or marked insulin resistance, has been reported to increase the androstenedione (62,63), but not the testosterone (62–64) or DHEAS level (64); in fact, decreases in testosterone (63) and DHEAS (64) and an increase in estradiol (63) have been reported. Hyperinsulinemia from glucose challenge similarly appears, if anything, to lower rather than raise androgen levels (65,66). That PCOS has been reported to occur without insulin resistance or hyperinsulinemia (67) suggests that the insulin resistance and hyperinsulinemia may not be responsible for the hyperandrogenemia of PCOS. In summary, while there is evidence in women that hyperandrogenemia may underlie hyperinsulinemia and insulin resistance, there is also evidence that hyperinsulinemia and insulin resistance may underlie hyperandrogenemia.

Whether the hyperandrogenemia observed in the diabetic subjects in the present study has a causal relationship to diabetes is also unclear. Lower fasting glucose and insulin levels in postmenopausal women taking estrogen suggest a favorable effect of estrogen on glucose metabolism (68), but ethinyl estradiol administered orally to premenopausal women appears to decrease insulin sensitivity (69). The effect of estrogen may depend on the estrogen preparation, dose, and route of administration. In postmenopausal women, oral equine estrogens have been reported to increase insulin sensitivity at a lower dose but decrease it at a higher dose (70), whereas transdermal estrogen at a dose similar to the higher dose increased insulin sensitivity (71).

In conclusion, type 2 diabetes in postmenopausal women was found to be associated with hyperandrogenemia and hyperestrogenemia. The hyperestrogenemia may have been secondary to the hyperandrogenemia. Evidence that hyperandrogenemia is associated with CHD and its risk factors in women raises the possibility that hyperandrogenemia may be a link between diabetes and CHD in women and may underlie both. If this is the case, lowering the androgen level in women may have a preventive effect on both diabetes and CHD.

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