Androgenicity and Obesity Are Independently Associated With Insulin Sensitivity in Postmenopausal Women

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An increase in androgenicity may contribute to the development of insulin resistance in postmenopausal women. Increased androgenicity in women has been found to be associated with the development of type 2 diabetes. In addition, obesity and central obesity are associated with greater androgenicity. Insulin sensitivity, androgenicity, and body composition were characterized in 34 nondiabetic postmenopausal women age 72 ± 1 years (mean ± SEM) to test the hypothesis that androgenicity is a predictor of insulin sensitivity independent of measures of obesity. Androgenicity was measured using levels of sex hormone-binding globulin (SHBG), total and free testosterone, dehydroepiandrosterone sulfate (DHEA-S), androstenedione, and free androgen index (FAI). Insulin sensitivity (S_I) was determined from a frequently sampled intravenous glucose tolerance test. Body composition measures included body mass index (BMI) and dual energy x-ray absorptiometry measurements of total and central fat mass. S_I was found to be associated with total fat mass ($r = -.51$, $P = .002$), central fat mass ($r = -.62$, $P = .0001$), BMI ($r = -.55$, $P = .0008$), SHBG levels ($r = .65$, $P = .0001$), and FAI ($r = -.41$, $P = .01$). SHBG levels were inversely correlated with central fat mass ($r = -.59$, $P = .0002$). Using multiple regression, SHBG and central fat mass were the only significant independent predictors of S_I, accounting for 50% of its variance ($r = .71$, $P = .0001$); total fat mass, BMI, total and free testosterone, DHEA-S, androstenedione, and FAI did not enter the model. We conclude that there is a significant association between insulin sensitivity and androgenicity in postmenopausal women that is independent of obesity. Interventions to decrease androgenicity may therefore be useful in improving insulin sensitivity in postmenopausal women.

ALTHOUGH AGING HAS been associated with insulin resistance, it appears that the age-associated increase in adiposity and blood pressure and decrease in aerobic capacity accounts for the majority of this association.[1] After menopause, although total body weight remains relatively constant, women lose lean body mass and muscle mass, while total body fat and central body adiposity increase.[2] Increased central body adiposity has been associated with skeletal muscle insulin resistance.[3] In addition to an increase in adiposity, postmenopausal women are characterized by a relative hyperandrogenicity because there is a greater decrease in estradiol and estrone after menopause than the decrease in the androgens, testosterone, and androstenedione. Androgenicity, the relative increase in androgen levels in the estrogen to androgen ratio, can be measured using levels of sex hormone-binding globulin (SHBG), total and free testosterone, dehydroepiandrosterone sulfate (DHEA-S), androstenedione, or the free androgen index (FAI). Increased androgenicity may also contribute to insulin resistance in postmenopausal women.

The relationship between androgenicity and insulin resistance is complex. Androgenicity has been found to be associated with increased fasting insulin levels[4] and with an increased risk of development of type 2 diabetes in women[5,6]), suggesting a relationship between increased androgenicity and insulin resistance. Further supporting a relationship between androgenicity and insulin resistance, the use of hormonal replacement therapy decreases androgenicity[7] and has been shown to improve insulin sensitivity in postmenopausal women.[8] In addition, an association between adiposity and androgenicity has been identified in postmenopausal women.[9] With increased abdominal adiposity, measured by waist-to-hip circumference ratio or the ratio of subscapular-to triceps skinfolds, there is greater androgenicity.[10] Although several studies have attempted to link obesity, androgenicity, and insulin sensitivity, these studies were limited to premenopausal women.[11,12]

The objective of this study was to test the hypothesis that androgenicity is a predictor of insulin sensitivity independent of measures of obesity in postmenopausal women. We report that androgenicity is a significant predictor of insulin sensitivity in postmenopausal women even when accounting for measures of central and total body adiposity.

MATERIALS AND METHODS

Subjects

Thirty-four healthy postmenopausal women (age range, 61 to 86 years) who were not on hormonal replacement therapy were studied. The ethnic background of these women included 33 Caucasian subjects, 1 American Indian subject, and 1 African American subject.

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Subjects were recruited through the Human Research Subject Participant Core of the University of Michigan Geriatrics Center, the University of Michigan Women's Health Registry, as well as through newspaper advertisement. Subjects were screened before study entry with a medical history, physical examination, laboratory tests, including a complete blood count, thyroid-stimulating hormone (TSH), routine chemistries, and an oral glucose tolerance test. Subjects were excluded from participation if they met criteria for diabetes by a 75-g oral glucose tolerance test[13] or had evidence from either history, physical exam, or laboratory testing of other significant underlying medical or psychiatric illness. Because SHBG levels can be affected by thyroid hormones, insulin, or cirrhosis, only subjects found to be euthyroid, nondiabetic, and to have normal liver function tests were included. All subjects were instructed not to engage in strenuous exercise for 24 hours prior to the frequently sampled intravenous glucose tolerance test (FSIVGTT) protocol. Each subject gave written informed consent that was approved by the University of Michigan Human Use Committee.

Measurement of Androgenicity

Plasma testosterone by chemiluminescence and plasma free testosterone, androstenedione, and DHEA-S by radioimmunoassay (RIA) were measured in the Core Laboratory of the University of Michigan General Clinical Research Center. Plasma SHBG levels were measured using a solid-phase, 2-site chemiluminescent enzyme immunometric assay for use with the Immulite Automated Analyzer (Immulite SHBG; Diagnostic Product., Los Angeles, CA). Plasma samples were collected during the baseline period of the FSIVGTT and stored at −70°C until assayed. The sensitivity of the SHBG assay was 0.2 nmol/L. The intra-assay and interassay coefficients of variation were 6.5% and 8.7%, respectively. SHBG levels are inversely related to the level of androgenicity. The FAI was calculated with the formula: 3.467 × total testosterone (ng/dL)/SHBG (nmol/L).[14]

Measurement of Insulin Sensitivity

A FSIVGTT was performed as described by Bergman[15] with the addition of insulin (0.02 U/kg intravenously over 30 seconds at 20 minutes) to enhance precision of the estimates of insulin action.[16] Subjects were instructed to consume a 200-g carbohydrate weight-maintaining diet for 3 days prior to the study. They then reported to the Clinical Research Center after an overnight (12-hour) fast and were studied in the supine position. An intravenous catheter was placed in the antecubital vein of 1 arm for the injection of glucose and insulin. Another catheter was placed in a retrograde manner into a dorsal hand vein of the contralateral arm, which was then placed into a warming box heated to 60°C to obtain arterialized-venous blood samples. Twenty minutes after the intravenous lines were inserted, 3 baseline samples for glucose and insulin were obtained at 5-minute intervals. At 0 minutes, 50% glucose (300 mg/kg) was given as an intravenous push over 30 seconds. Blood samples were collected at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes after the glucose bolus.

Measurements of Body Composition

Lean body mass and total body composition were determined by DEXA[17] (model DPX-L; Lunar Radiation, Madison, WI). The DEXA measure of abdominal adiposity (DXA L1/L4) or central fat mass was determined with the manual analysis component of the Lunar software package (Lunar software version 4.5c, extended research analysis). A rectangle was drawn on the digital scan image bounded superiorly by the horizontal line identifying the T12/L1 intervertebral space, inferiorly by the horizontal line denoting the L4/L5 intervertebral space, and bilaterally by connecting the 2 horizontal lines in a region free of tissue. Abdominal adiposity, DXA L1/L4, was defined as the fat mass within this region.

Table 1. Subject Characteristics

	Mean ± SEM	Range
Age (yr)	72 ± 1	61-86
Weight (kg)	68 ± 2.2	45-99
Body mass index (kg/m^2)	26.1 ± 0.75	17.8-37.1
Fasting glucose (mg/dL)	101 ± 1.5	82-116
Fasting insulin (μU/mL)	12 ± 0.9	5-24
Total body fat (%)	39 ± 1.6	16-53
Central fat mass (g)	2545 ± 217	411-5820
S_I ($\times 10^{-4}$/min/μU/mL)	3.3 ± 0.3	0.8-7.9
SHBG (nmol/L)	53.2 ± 5	19.7-126.5
Total testosterone (ng/mL)	0.17 ± 0.01	0.06-0.35
Free testosterone (pg/mL)	0.57 ± 0.06	0.20-1.70
Androstenedione (ng/mL)	0.89 ± 0.05	0.4-1.40
DHEA-S (μg/dL)	58.2 ± 6.6	10-158

NOTE. n = 34.

Data and Statistical Analysis

Blood samples for plasma glucose and insulin were collected into chilled glass tubes containing sodium heparin, stored on ice and separated immediately following each study. Plasma was stored at −70°C until assay. Plasma glucose was measured by the Autoanalyzer glucose oxidase method and plasma insulin by RIA in the Core Laboratory of the Michigan Diabetes Research and Training Center. The S_I (sensitivity to insulin index) was calculated from a least-squares fitting of the temporal pattern glucose and insulin throughout the FSIVGTT using the MINMOD program.[15]

Values are presented as means ± SEM. Body mass index (BMI, kg/m^2) was determined by the subject's weight divided by the subject's height squared. Central fat mass and total fat mass were measured by DEXA. The relationships between S_I, androgenicity, and central fat mass were analyzed using univariate linear regression. Stepwise multiple regression models were constructed using Mallow's C(p) criterion for the dependent variable S_I and the independent variables that were statistically significant by univariate analysis. Statistical analysis was performed using SAS (SAS, Cary, NC). A value of $P \leq .05$ was selected to indicate statistical significance.

RESULTS

Subject Characteristics

The subject characteristics are described in Table 1.

Univariate Relationships

As expected, insulin sensitivity was lower in those with greater obesity (BMI and total fat, Table 2, as well as central adiposity, Fig 1). Significant correlations were found between SHBG levels and measures of adiposity (Table 2) including central fat mass ($r = -.59$, $P = .0002$, Fig 2). There were also significant associations found between fasting insulin levels and SHBG levels ($r = -.53$, $P = .0014$, Table 2), as well as between insulin sensitivity and SHBG levels ($r = .65$, $P = .0001$, Fig 3).

Although there were no significant associations found between total and free testosterone with adiposity or insulin sensitivity, there was a significant association found between

Table 2. Correlation Matrix Between S_I and Baseline Values for Insulin and Measures of Adiposity and Androgenicity

	Fasting Insulin	BMI	Total Fat Mass	Central Fat Mass	SHBG	Free Testosterone	Total Testosterone	Free Androgen Index	DHEA-S	Androstenedione
S_I	−0.70*	−0.55*	−0.51*	−0.62*	0.65*	−0.29	0.05	−0.41*	−0.13	−0.13
Fasting insulin		0.42*	0.37*	0.44*	−0.53*	0.21	−0.12	0.23	0.05	0.05
BMI			0.95*	0.89*	−0.52*	0.32	0.005	0.34*	0.18	0.08
Total fat mass				0.91*	−0.51*	0.29	−0.03	0.32	0.09	0.02
Central fat mass					−0.59*	0.29	−0.02	0.34*	0.14	0.06
SHBG						−0.14	0.14	−0.55*	0.008	0.02
Free testosterone							0.73*	0.66*	0.63*	0.67*
Total testosterone								0.61*	0.53*	0.60*
Free androgen index									0.30	0.30
DHEA-S										0.75*
Androstenedione										

Abbreviation: BMI, body mass index.
*$P < .05$ by univariate analyses.

the FAI and insulin sensitivity ($r = -.41$, $P = .01$, Table 2). There were no significant associations found between the adrenal androgens (DHEA-S, androstenedione) and insulin sensitivity or measures of obesity (Table 2).

Multivariate Regression Models

To test whether androgenicity would be an independent predictor of insulin sensitivity even though it was highly correlated with central adiposity, multiple regression analysis was used. The dependent variable in the model was insulin sensitivity. The independent variables tested in the model were the variables that had significant univariate relationships: SHBG, FAI, BMI, central fat mass, and total fat mass. The model selection method used was a stepwise method using Mallow's C(p) criterion, which chooses independent variables that best describe the variance, but also accounts for the number of independent variables in the model. The best model for predicting insulin sensitivity included SHBG and central fat mass, together accounting for 50% of the variance in S_I ($P = .0001$). SHBG was the independent variable that entered the model first explaining 42% of the variance in S_I ($P = .009$) with central fat mass explaining an additional 8% of the variance in S_I ($P = .03$) when added to the model. FAI, BMI, and total fat mass did not enter into this model. Because the calculation of FAI involves SHBG, SHBG was removed from the model to determine if other androgenicity measures would remain significantly associated with insulin sensitivity. The best model for predicting S_I excluding SHBG as an independent variable included central fat mass, FAI, and total testosterone, together accounting for 48% of the variance in S_I ($P = .0002$). Central fat mass was the independent variable that entered this model first, explaining 37% of the variance in S_I ($P = .007$) with FAI explaining an additional 5% of the variance in S_I ($P = .01$) and total testosterone explaining an additional 6% of the variance in S_I ($P = .02$). Detailed results from these 2 multiple regression models are summarized in Table 3. Using the stepwise method, the relationship between androgenicity and insulin sensitivity remained significant in subgroups of normal and overweight subjects. In those subjects with BMI < 26, the best model for predicting S_I was the FAI alone accounting for 25% of the

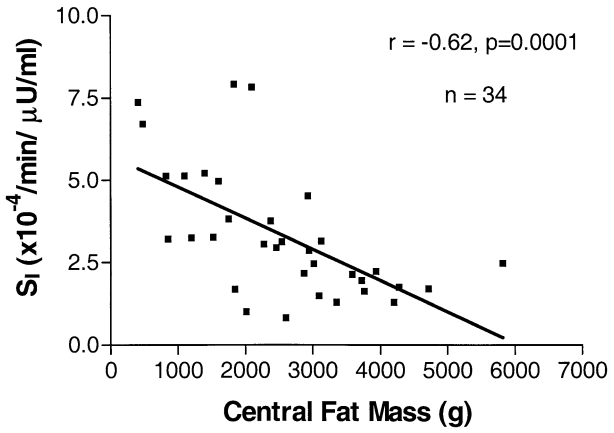

Fig 1. Univariate association between central fat mass and S_I. Insulin sensitivity is lower in those with greater fat mass, $r = -.62$, $P = .0001$.

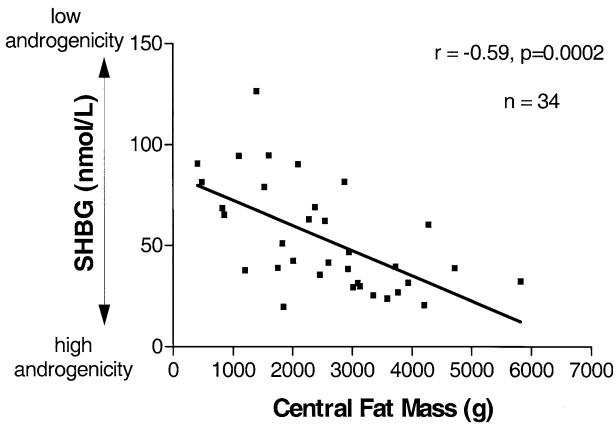

Fig 2. Univariate association between SHBG and central fat mass. SHBG is significantly correlated with central fat mass, $r = -.59$, $P = .002$.

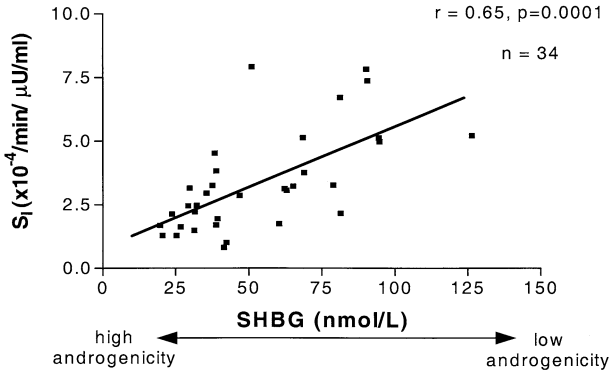

Fig 3. Univariate association between SHBG and S_I. Insulin sensitivity is lower in those with greater androgenicity as measured by SHBG levels, $r = .65$, $P = .0001$.

variance in S_I ($P = .05$). In those subjects with BMI ≥ 26, the best model for predicting S_I was SHBG alone accounting for 35% of the variance in S_I ($P = .01$). Measures of obesity were not significantly associated with androgenicity in either the normal or overweight subgroups.

DISCUSSION

The results of this study in a group of healthy nondiabetic postmenopausal women not receiving hormonal replacement therapy are consistent with previous studies that demonstrate: (1) increased insulin resistance with increased total and central adiposity, (2) increased fasting insulin levels with increased androgenicity, and (3) increased androgenicity with increased total and central adiposity. In addition, the major new finding is that the significant independent association between insulin resistance and androgenicity persists even when accounting for measures of obesity.

As expected, in those subjects with greater BMI, total, and central fat mass, insulin sensitivity was lower. After menopause, women undergo changes that are associated with an increased risk for insulin resistance. One of these changes is the increase in central body adiposity that have been shown to be accelerated after menopause. In a longitudinal study by Poehlman et al,[2] women who experienced menopause had a greater increase in fat mass and waist-to-hip ratio compared with women who remained premenopausal. Increases in central body adiposity have been found to be associated with adverse metabolic consequences, including insulin resistance, hypertriglyceridemia, increased mortality from coronary artery disease, and increased atherothrombotic events.[3]

Postmenopausal women also undergo changes in their hormonal profile, developing a relative hyperandrogenicity. After menopause, there is a greater decrease in estrogens, estradiol, and estrone, relative to the decrease in androgens, testosterone, and androstenedione. The mean SHBG level in our study population was 53 ± 4 nmol/L, comparable to the lower ranges of SHBG values (mean SHBG value of 55 ± 31 nmol/L) in the prospective study by Linstedt et al.[5] In the Linstedt study, SHBG levels were tested in a representative cross section of women and after 12 years of follow-up, these women were evaluated for the development of diabetes. The women with lower SHBG values (indicating greater androgenicity) were found to have an increased incidence of diabetes mellitus. Similar findings have been found using different measurements of androgenicity. Oh et al[18] showed that high testosterone levels predicted development of type 2 diabetes in older women. In our study, greater androgenicity (as evidenced by lower SHBG levels and higher FAI) was associated with increased fasting insulin levels and lower insulin sensitivity. There also was an association found between SHBG levels and measures of obesity so that in those with lower SHBG levels, BMI, total, and central fat mass were greater. This association has been previously reported in premenopausal women,[19] as well as postmenopausal women.[10,20]

The association between SHBG levels (as a measure of androgenicity) and insulin sensitivity and the association between adiposity and insulin sensitivity were identified with univariate analyses. Both were found to have significant linear relationships with insulin sensitivity, such that insulin sensitivity was lower in those with greater fat mass and lower in those with greater androgenicity. These individual relationships between adiposity, insulin sensitivity, and androgenicity have been reported previously in premenopausal women. There have also been studies in premenopausal women demonstrating a relationship between all three. The findings reported in these studies have not been consistent with one study suggesting that obesity leads to change in SHBG levels leading to insulin resistance,[12] while the other study suggests that hyperandrogenicity may be an additional determinant of hyperinsulinemia in obese women.[11]

To test the hypothesis that androgenicity would remain significantly associated with insulin sensitivity in this group of postmenopausal women, even though insulin sensitivity was highly correlated with adiposity, multiple regression modeling

Table 3. Multiple Regression Analyses for Dependent Variable S_I With (Model 1) and Without SHBG (Model 2)

	Independent Variable	β (SE)	T Statistic	*P* Value
Model 1	SHBG	0.032 (0.012)	2.797	.009
n = 34, R^2 = .50	Central fat mass	−0.0005 (0.0002)	−2.256	.03
Model 2	Central fat mass	−0.0006 (0.0002)	−2.900	.007
n = 34, R^2 = .48	FAI	−0.965 (0.348)	−2.770	.01
	Total testosterone	12.155 (4.883)	2.489	.02

Abbreviation: FAI, free androgen index.

was performed. In addition to the significant associations between adiposity and androgenicity, both were independently associated with insulin sensitivity. These associations were consistent using different measures of androgenicity, either with SHBG or when SHBG was excluded from the analysis, with total testosterone and FAI. Additional analyses included forcing either adiposity or androgenicity into a model that included the variables SHBG, BMI, central fat mass, and total fat mass; both adiposity and androgenicity remained significant independent predictors of insulin sensitivity. In subjects subgrouped as normal or overweight, androgenicity remained a significant predictor of insulin sensitivity. No interaction was found between androgenicity and adiposity. These findings contrast with a recent study in younger, moderately obese, nondiabetic men in whom SHBG levels were not found to be independently related to glucose disposal rate after accounting for measures of adiposity.[21] This suggests that the relationships between insulin sensitivity, adiposity, and androgenicity may differ by sex, age, and degree of obesity and warrant further studies.

There is evidence that estrogen replacement therapy in women may modify postmenopausal changes and decrease the risk of developing insulin resistance. Many animal studies,[22-24] epidemiologic data,[25,26] and some prospective human studies[8,27-33] have reported on the effect of estrogen on insulin sensitivity. Overall, estrogen appears to increase insulin sensitivity. One potential mechanism by which estrogen may improve insulin sensitivity is by preventing the increase in central adiposity after menopause. A subgroup analysis of the subjects in the Postmenopausal Estrogen/Progestin Interventions trial demonstrated that women randomized to any active hormone replacement therapy gained less weight and did not have as great of an increase in waist or hip girth compared with women on placebo, even when age, overall activity, ethnicity, smoking status, and alcohol consumption were controlled for.[34] Haarbo et al[35] also demonstrated that healthy postmenopausal women treated with hormone replacement therapy for up to 2 years maintained their percent abdominal fat constant as measured by DEXA compared with a significant increase in abdominal fat in healthy postmenopausal women treated with placebo, suggesting that hormone replacement therapy prevents the increase in central adiposity that occurs after menopause. Another potential mechanism by which estrogen may improve insulin sensitivity is by decreasing androgenicity, as measured by increasing SHBG levels. Oral conjugated equine estrogens have been shown to induce the liver's synthesis of SHBG at doses as low as 0.3 mg/d.[7] Thus, estrogen appears to decrease the development of central adiposity after menopause, decrease androgenicity, and may improve insulin sensitivity. Changes in estrogen or androgenicity may therefore play a role in the development of insulin resistance in this group.

We acknowledge several potential limitations inherent in our study. First, these results are cross-sectional associations, and the causal relationships between androgenicity and insulin sensitivity remain undefined. There is evidence that insulin levels may directly regulate SHBG levels. In vitro, insulin decreased SHBG production by human hepatoma cells.[36] In vivo, in women with insulin-resistant states, changes in insulin levels led to changes in SHBG levels.[37,38] It is unclear whether these findings are applicable to healthy postmenopausal women. Second, we recognize that using DEXA to measure body composition and central obesity does not differentiate between subcutaneous and visceral fat. However, this method has been found to be a reliable and reproducible measure of absolute fat mass compared with abdominal computed topography (CT) measures of visceral adiposity.[39] Third, although SHBG and central fat mass accounted for 50% of the variance in S_I, we did not include other important determinants (aerobic capacity, blood pressure, etc) of insulin sensitivity. Therefore, we are unable to estimate the variance in S_I that might be explained by these other measures relative to the contribution of SHBG.

In conclusion, in postmenopausal women, there is a significant association between androgenicity and insulin sensitivity that is independent of obesity and central adiposity. Based on these results, in addition to weight loss, interventions to decrease androgenicity may also be useful to improve insulin sensitivity in this group. Future prospective studies would be helpful to determine causality and also to examine whether these relationships are also found in older men.

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