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Association of Endogenous Sex Hormones and Insulin Resistance among Postmenopausal Women: Results from the Postmenopausal Estrogen/Progestin Intervention Trial

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Most studies of sex hormones and insulin resistance (IR) have focused on androgens; few have examined the association of endogenous estrogens and IR. We determined the cross-sectional association of endogenous levels of total and bioavailable testosterone and estradiol and SHBG with IR among 845 healthy, postmenopausal women aged 45–65 yr. Women were within 10 yr of menopause and not using hormone replacement therapy. Total adiposity was estimated by body mass index, visceral adiposity by waist to hip ratio (WHR), and IR by the homeostasis model assessment. We defined homeostasis model assessment-IR as the highest quartile (cutpoint, 2.1) of the distribution in this cohort. In logistic regression analyses, the odds for IR were significant and increased in a dose-response fashion across each quartile of total estradiol, bioavailable estradiol, and bioavailable testosterone (all $P < 0.001$ for linear trend). These associations remained significant after adjusting for WHR; adjusted odds ratios were 4.0, 6.1, and 2.7 for total estradiol, bioavailable estradiol, and bioavailable testosterone, respectively, comparing the highest to the lowest quartile (all $P < 0.001$). Adjusting for body mass index and WHR together eliminated the linear association of IR with total estradiol and bioavailable testosterone, but the association with bioavailable estradiol remained (adjusted odds ratio, 2.7; $P < 0.001$, comparing the highest to the lowest quartile). IR was not associated with total testosterone before or after adjusting for adiposity. Lower SHBG levels were associated with higher odds of IR, independent of adiposity. These results suggest that estrogen may be equally or more important than testosterone in the pathway to IR in healthy, young postmenopausal women, with differences not entirely explained by body size. (*J Clin Endocrinol Metab* 88: 1646–1652, 2003)

THE MENOPAUSE TRANSITION is marked by changes in hormonal balance, including a rapid decline of endogenous estradiol levels, leading to a period of relative androgen excess (1). It has been suggested that this shift in hormonal balance contributes to an increase in visceral adiposity (2) that is associated with insulin resistance (IR) in postmenopausal women (3).

Although the association between bioavailable (non-SHBG bound) testosterone and IR or incident diabetes among postmenopausal women has been reported previously (4–8), studies of the association between endogenous estradiol levels and IR are limited. Two small studies (n = 75 and 20) reported associations between total estradiol and fasting insulin among apparently healthy postmenopausal women (7, 9). Another small study reported higher total estradiol levels among 20 Hispanic postmenopausal women with diabetes compared with 29 euglycemic women (4). Bioavailable estradiol has been shown to be positively associated with impaired glucose tolerance (6) and the development of IR and type 2 diabetes (5) in an elderly population whose mean age was 72 yr. To our knowledge, no large studies have examined the association of IR with total or bioavailable estradiol among young, healthy postmenopausal women.

We report here the cross-sectional association of total and bioavailable endogenous sex hormones and SHBG levels with IR in 845 healthy, postmenopausal women aged 45–65 yr. The impact of total adiposity, as measured by body mass index (BMI), and visceral adiposity, estimated by the waist to hip ratio (WHR), on these associations was evaluated. Women were within 10 yr of menopause and were not using hormone replacement therapy.

Abbreviations: BMI, Body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment; IR, insulin resistance; PCOS, polycystic ovary syndrome; WHR, waist to hip ratio.

Subjects and Methods

Design and overview

Population. Study participants were women in the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial, described previously (10, 11). The present analysis uses data obtained at baseline before study interventions were begun. Between December 1989 and February 1991, 875 healthy postmenopausal women between the ages of 45–65 yr from seven clinical centers in the United States were enrolled in a randomized clinical trial. Key eligibility criteria at enrollment included the following: 1) at least one and not more than 10 yr postmenopausal; 2) serum FSH at least 40 mIU/ml for women who had undergone hysterectomy before menopause; 3) no hysterectomy within 2 months; 4) no medical history of insulin-dependent diabetes; 5) fasting plasma glucose (FPG), 140 mg/dl or less; 6) BMI, 40 kg/m^2 or below; and 7) no hormone replacement therapy within 3 months.

Glucose and insulin tests. A standard 75-g oral glucose tolerance test was performed between 0700 and 1100 h after a 12-h overnight fast; blood

was obtained by venipuncture at 0, 60, and 120 min. Samples were shipped frozen to the Central Glucose Insulin Laboratory (CGIL) at the Indiana University School of Medicine for analysis. Plasma glucose was measured using a colorimetric glucose oxidase method after Somogyi precipitation (12). The interassay coefficients of variation, based on Boehringer Mannheim Diagnostics control pools, were 2.0 and 1.8% for glucose values of 3.94 and 16.10 mmol/liter, respectively. A modified double antibody method was used to measure serum insulin. Interassay coefficients of variation were 29, 14, and 13% for values of 49, 297, and 646 pmol/liter, respectively, based on Bio-Rad control pools; assay sensitivity was between 7 and 14 pmol/liter.

Homeostasis model assessment (HOMA) for IR (HOMA-IR) was used to measure insulin resistance; HOMA-IR calculations allow determination of IR based on fasting glucose and insulin measurements [R = insulin (mU/liter)/22.5$e^{-\ln \text{glucose (mmol/liter)}}$ or R = (insulin × glucose)/22.5) in simplified form]. HOMA-IR correlates strongly with euglycemic hyperinsulinemic clamp techniques in normoglycemic (R_s = 0.83; $P < 0.001$) and diabetic subjects (R_s = 0.92; $P < 0.001$; Ref. 13) and is an appropriate method for evaluating IR in large populations (14). HOMA and fasting insulin measurements were highly correlated in the present study (r = 0.99), similar to the correlation between HOMA and fasting insulin in other studies (15).

Sex hormones and SHBG. Sex hormones and SHBG were measured in the University of California-San Diego Reproductive Endocrinology research laboratory. Total estradiol and testosterone levels were measured by RIA after organic solvent extraction and celite column chromatography. Bioavailable (non-SHBG bound) levels of estradiol and testosterone were determined by a modified ammonium-sulfate precipitation method (16). SHBG was measured by the method of Rosner (17). Sensitivity, intra- and interassay coefficients, respectively, were 0.07 nmol/liter, 4%, and 4.9% for testosterone; 11.0 pmol/liter, 5.9%, and 7.1% for estradiol; 0.02 nmol/liter, 6.5%, and 10.7% for bioavailable testosterone; 5.5 pmol/liter, 6.1%, and 7.9% for bioavailable estradiol; and 0.10×10^{-8} mol/liter, 7.5%, and 8.0% for SHBG. (Conversion factors: estradiol, picomoles per liter divided by 3.671 yields picograms per milliliter; testosterone, nanomoles per liter divided by 3.467 yields nanograms per milliliter.) In this study, 66 women had total and bioavailable estradiol levels below the assay sensitivity; values just below the assay sensitivity were assigned for these analyses (7.3 and 1.8 pmol/liter, respectively, equivalent to 1 pg/ml below the respective assay sensitivities). Total and bioavailable testosterone levels were within the assay range for all women. Nine women were excluded from this analysis because of missing hormone levels. An additional 21 women who had total estradiol levels exceeding 2 SD values above the mean (suggesting unreported exogenous estrogen use) were excluded, leaving a study population of 845.

Clinical evaluation. Height, waist girth, and hip girth were measured to the nearest 0.5 cm. Weight and height were measured with participants wearing light street clothes and no shoes; waist was determined as the smallest circumference between the ribs and iliac crest. Hip girth was determined as the largest circumference around the buttocks.

Demographic parameters and lifetime history of cigarette smoking and alcohol consumption were determined using standardized questionnaires. Participants were asked the number of days per month that they drank alcoholic beverages and how many drinks they consumed on these days. Participants were asked whether they regularly engaged in strenuous exercise (such as jogging, singles tennis, paddleball, or high-intensity aerobics) or hard physical labor at least three times a week.

The PEPI protocol and consent forms were approved by the institutional review board at each clinical center; all participants gave written informed consent.

Statistical analysis

Data were analyzed using SAS (version 8.0, SAS Institute, Inc., Cary, NC) and SPSS (version 10.1, SPSS, Inc., Chicago, IL) software. Descriptive statistics were calculated. Spearman correlations were calculated for sex hormones, SHBG, and HOMA-IR with other metabolic, hormone, and anthropometric variables. HOMA-IR values were calculated across quartiles of sex hormone levels and SHBG, and a linear test of trend was performed. HOMA-IR values were also determined within tertiles of WHR in each tertile of bioavailable estradiol, bioavailable testosterone, and SHBG. IR was defined as the highest quartile of HOMA-IR, as published previously (15). Logistic regression analysis was used to evaluate the odds of being insulin resistant within categories of sex hormones and SHBG, with and without adjustment for measures of adiposity. Regression models were compared using likelihood ratio tests. Odds ratios for sex hormones and SHBG quartiles were tested for linear trends.

Results

The 845 women in this study had an average age of 56.2 yr (range, 46–65 yr), an average BMI of 25.9 kg/m^2 (range, 17–40 kg/m^2), an average waist girth of 81.8 cm (range, 61–123 cm), and an average WHR of 0.79 (range, 0.59–1.13). Only 13.4% were current smokers, 26.3% drank alcohol more than three times a week, 23.2% reported strenuous physical activity at least three times a week, 31.6% had undergone a hysterectomy, and 6.9% reported bilateral oophorectomy. Twenty-eight women had diabetes based on FPG levels of at least 126 mg/dl (n = 9) and/or 2-h postchallenge glucose levels of at least 200 mg/dl (n = 21), 62 women had impaired fasting glucose (FPG ≥ 110 mg/dl and < 126 mg/dl), and 129 women had impaired glucose tolerance (2-h postchallenge glucose between 140 mg/dl and 200 mg/dl); all were included in this study for comparability with other studies that used these criteria. Table 1 presents the distribution of metabolic and sex hormone-related variables.

Univariate associations of clinical characteristics, endogenous sex hormones, and SHBG levels with HOMA-IR are shown in Table 2. Total and bioavailable estradiol and bioavailable, but not total, testosterone were positively associated with HOMA-IR; SHBG was inversely associated with HOMA-IR. Adjustment for age did not change the significance of these associations. HOMA-IR was correlated with fasting insulin (r = 0.99), 2-h postchallenge insulin (r = 0.57), fasting glucose (r = 0.40), and 2-h postchallenge glucose (r = 0.29; all $P < 0.001$). As shown, BMI, waist girth, and WHR were positively associated with bioavailable testosterone and total and bioavailable estradiol and negatively associated with SHBG.

Figure 1 shows mean HOMA-IR values by quartiles of sex hormones and SHBG. Increasing quartiles of total and bioavailable estradiol and bioavailable testosterone were significantly ($P < 0.001$) associated with an increase in mean HOMA-IR; total testosterone was not. Increasing SHBG quartiles were associated ($P < 0.001$) with a decrease in mean HOMA-IR. As shown in three-dimensional plots in Fig. 2, the

TABLE 1. Sex hormones, SHBG, and metabolic characteristics for 845 postmenopausal women

	Mean	SD	Interquartile range
Total testosterone (nmol/liter)	0.50	0.25	0.33–0.63
Bioavailable testosterone (nmol/liter)	0.11	0.08	0.06–0.14
Total estradiol (pmol/liter)	23.5	26.9	15–22
Bioavailable estradiol (pmol/liter)	11.0	13.3	4–11
SHBG (nmol/liter)	44.7	23.4	28–57
HOMA-IR	1.7	1.6	0.6–2.1
Fasting insulin (pmol/liter)	48.1	43.7	18–63
2-h insulin (pmol/liter)	431.5	484.3	196–488
Fasting glucose (mmol/liter)	5.4	0.6	5.1–5.6
2-h glucose (mmol/liter)	6.3	2.0	5.0–7.2

TABLE 2. Spearman correlation coefficients of clinical characteristics, endogenous sex hormone levels, and SHBG with HOMA-IR[a]

	BMI	Waist girth	WHR	Testosterone	Bioavailable testosterone	Estradiol	Bioavailable estradiol	SHBG	HOMA-IR
Age	NS	NS	0.15	NS	NS	−0.12	−0.13	NS	NS
BMI		0.85	0.42	NS	0.34	0.44	0.52	−0.45	0.46
Waist girth			0.72	NS	0.34	0.41	0.51	−0.48	0.50
WHR				NS	0.25	0.22	0.34	−0.41	0.41
Fasting insulin				NS	0.23	0.22	0.31	−0.32	0.99
2-h insulin				NS	0.25	0.20	0.33	−0.40	0.57
Fasting glucose				NS	0.19	0.15	0.26	−0.34	0.40
2-h glucose				NS	0.21	0.17	0.26	−0.32	0.29
Testosterone					0.69	0.39	0.25	0.13	NS
Bioavailable testosterone						0.50	0.59	−0.50	0.24
Estradiol							0.86	−0.29	0.23
Bioavailable estradiol								−0.58	0.33
SHBG									−0.35

NS, Nonsignificant.
[a] All correlation coefficients shown significant at $P < 0.001$.

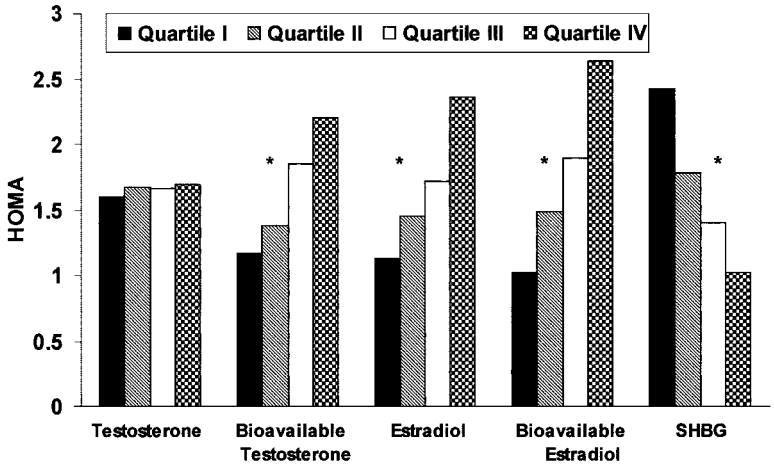

FIG. 1. Unadjusted mean HOMA-IR values by quartiles of sex hormones and SHBG. *, $P < 0.001$ for linear trend.

relationship of bioavailable sex hormones and SHBG with HOMA-IR was most apparent among the highest tertiles of WHR.

Table 3 shows the results of logistic regression analyses relating IR to increasing quartiles of sex hormone levels and SHBG concentrations. IR was defined as the highest quartile of HOMA-IR (the cutpoint was 2.1 and the mean value for the IR quartile was 3.8 ± 1.9). BMI was used as a measure of overall adiposity, and WHR as an estimate of central adiposity. The odds for IR increased with each increasing quartile of total and bioavailable estradiol ($P < 0.001$ for linear trend) and, in both cases, the odds remained significant after adjusting for WHR ($P < 0.01$ for linear trend). Adjusting for BMI, or BMI and WHR together, eliminated the significant association of IR with total estradiol and restricted the association with bioavailable estradiol to the two highest quartiles.

IR was not associated with levels of total testosterone with or without adjustment for adiposity. Bioavailable testosterone had a linear association with IR before and after adjusting for WHR, but not after adjusting for BMI. Higher SHBG levels were associated with lower odds of being insulin resistant, and the linear trend remained significant after adjustment for adiposity measures. The associations of sex hormones and SHBG with IR were not materially changed when age, exercise and smoking status, and alcohol consumption were added to the model as covariates, nor were they changed when the logistic regression analysis was repeated, excluding 28 participants who had undiagnosed diabetes at baseline (data not shown).

Discussion

In this large sample of relatively young postmenopausal women without known diabetes, endogenous levels of total and bioavailable estradiol were significantly associated with insulin resistance, and these associations were independent of central adiposity as assessed by WHR. Adjustment for BMI, an estimate of total adiposity, eliminated the association of IR with total estradiol levels; however, bioavailable estradiol levels continued to predict IR. Measures of androgenicity (high bioavailable testosterone and low SHBG) were also associated with increased odds of being insulin resistant, and these results were independent of BMI and WHR.

This is the first large study to report a cross-sectional association between total and bioavailable estradiol and IR, and it supports results from the Rancho Bernardo study of older postmenopausal women showing cross-sectional associations between bioavailable estradiol levels and impaired glucose tolerance (6), as well as a prospective association between bioavailable estradiol and follow-up IR (5). In the present study, the odds for IR among quartiles of total

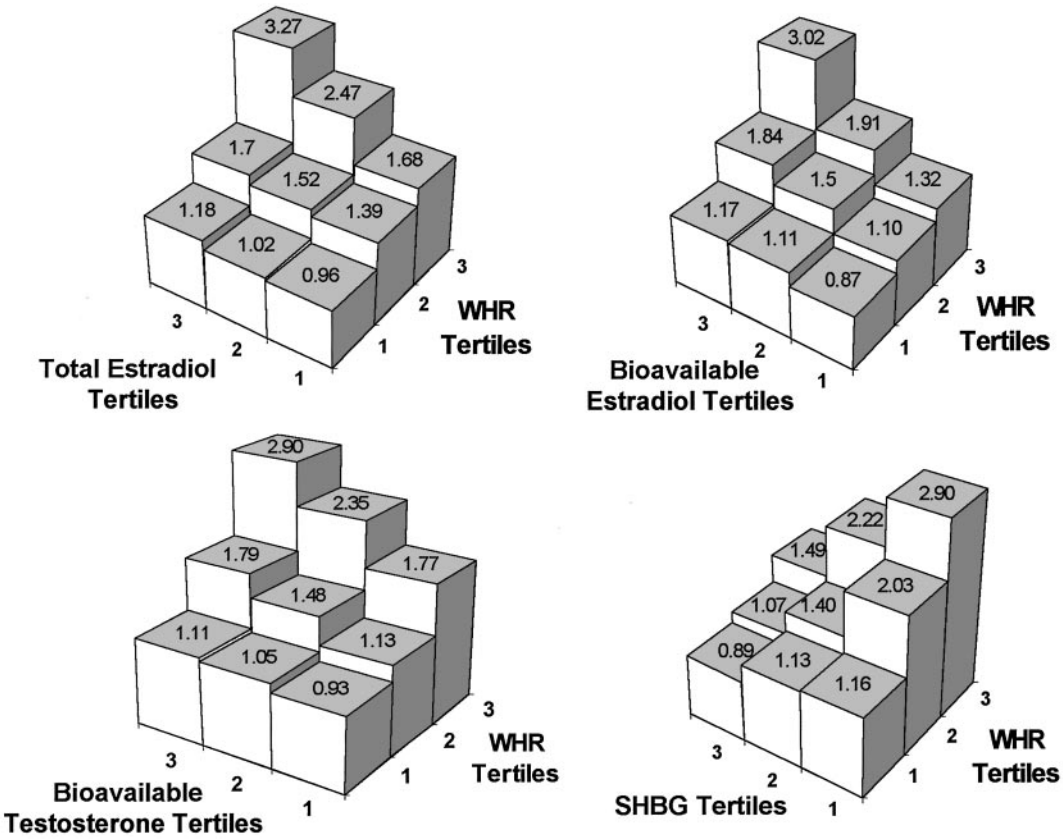

FIG. 2. Relationship of HOMA-IR to tertiles of WHR and tertiles of sex hormones and SHBG. Mean HOMA-IR values are presented at the *top* of each *column*.

and bioavailable estradiol were lowered after adjusting for BMI and WHR. This is not surprising, given the shift from ovarian to peripheral adipocyte production of estradiol after menopause. However, BMI and WHR did not completely explain the increased odds of IR observed among women in the highest quartiles of total and bioavailable estradiol, suggesting that estradiol is an independent marker of IR.

Neither the mechanism nor the direction of the association for the estradiol and IR relationship is understood. Although insulin has been shown to increase aromatase activity in some studies of women with polycystic ovary syndrome (PCOS) (18), these results are likely due to ovarian effects not relevant to postmenopausal women. We are unaware of studies reporting the effect of insulin infusion on endogenous estradiol production in postmenopausal women. The reverse causal direction is suggested by a recent study that showed the existence of nonclassical estrogen receptors on the plasma membrane of pancreatic β-cells (19); in this report, estrogen was found to act synergistically with glucose to enhance insulin secretion. Whether this estradiol-induced secretion of insulin can lead to sustained hyperinsulinemic IR is unknown. The use of exogenous estradiol by postmenopausal women has been shown to have both beneficial (20–22) and detrimental (23) effects on glucose metabolism. The differences in type, route, and dose of estrogen therapy, as well as different methods of assessing IR, may account for these discrepancies. In PEPI, hormone regimens decreased fasting glucose and increased postchallenge glucose with modest parallel changes in insulin levels (23).

In contrast to the limited research on endogenous estradiol and IR, the relationship between androgenicity and IR has been examined extensively, especially among women with PCOS. The coexistence of upper abdominal adiposity, androgenicity, and IR (24) among these women, in addition to the well known association between android patterns of obesity and IR among healthy women (25–27), has encouraged investigators to examine possible mechanisms that may explain the androgen-IR relationship. One possible mechanism is that insulin may stimulate ovarian androgen production as demonstrated *in vitro* (28), but insulin infusion studies conducted in women with PCOS have produced conflicting results [reviewed by Poretsky *et al.* (18) and Nestler (29)], possibly due to small samples, short durations of infusion, and limited ability to replicate physiological concentrations and diurnal variations of insulin. Reduction of circulating insulin in women with PCOS after treatment with metformin or diazoxide has consistently resulted in decreased androgens (30), suggesting that extended insulin exposure may stimulate androgen production over long durations. Whether insulin stimulates androgen production by the postmenopausal ovary is unknown.

If insulin does, indeed, have a stimulatory effect on ovarian androgen production, a parallel association between both total and bioavailable testosterone and IR would be expected.

TABLE 3. Odds ratios (95% CI) from logistic regressions relating HOMA-IR[a] to quartile of sex hormone and SHBG levels

	Unadjusted	Adjusted for BMI	Adjusted for WHR	Adjusted for BMI and WHR
Quartiles of total testosterone (nmol/liter)				
1 (≤0.33)	1.0	1.0	1.0	1.0
2 (0.34–0.46)	1.0 (0.6–1.5)	0.9 (0.5–1.4)	1.0 (0.6–1.7)	0.9 (0.6–1.6)
3 (0.47–0.63)	1.0 (0.6–1.5)	1.0 (0.6–1.6)	1.1 (0.7–1.7)	1.1 (0.7–1.9)
4 (≥0.64)	0.9 (0.5–1.3)	0.7 (0.4–1.1)	0.9 (0.6–1.4)	0.7 (0.4–1.2)
BMI		1.3 (1.2–1.3)[d]		1.2 (1.2–1.3)[d]
WHR			1.1 (1.2–1.3)[d]	1.1 (1.05–1.1)[d]
P value for linear trend	0.50	0.38	0.52	0.46
Quartiles of bioavailable testosterone (nmol/liter)				
1 (≤0.06)	1.0	1.0	1.0	1.0
2 (0.07–0.09)	1.7 (1.0–2.8)	1.3 (0.7–2.3)	1.4 (0.8–2.5)	1.2 (0.7–2.2)
3 (0.10–0.14)	3.0 (1.9–5.0)[d]	2.1 (1.2–3.6)[c]	2.5 (1.5–4.2)[c]	2.0 (1.1–3.4)[b]
4 (≥0.15)	3.8 (2.3–6.2)[d]	1.8 (1.1–3.2)[b]	2.7 (1.6–4.6)[d]	1.7 (1.0–2.9)[b]
BMI		1.3 (1.2–1.3)[d]		1.2 (1.2–1.3)[d]
WHR			1.1 (1.09–1.14)[d]	1.1 (1.05–1.10)[d]
P value for linear trend	<0.001	0.17	0.007	0.22
Quartiles of total estradiol (pmol/liter)				
1 (≤11)	1.0	1.0	1.0	1.0
2 (11.1–18.3)	2.4 (1.4–4.0)[c]	1.8 (1.0–3.2)[b]	1.9 (1.1–3.4)[b]	1.7 (0.9–3.0)
3 (18.4–22.0)	2.7 (1.6–4.6)[d]	1.5 (0.8–2.6)	2.1 (1.2–3.6)[c]	1.3 (0.8–2.4)
4 (≥22.1)	6.0 (3.6–10.1)[d]	1.9 (1.0–3.4)[b]	4.0 (2.3–6.9)[d]	1.7 (0.9–3.1)
BMI		1.3 (1.2–1.3)[d]		1.2 (1.2–1.3)[d]
WHR			1.1 (1.08–1.14)[d]	1.1 (1.05–1.10)[d]
P value for linear trend	<0.001	0.87	0.003	0.91
Quartiles of bioavailable estradiol (pmol/liter)				
1 (≤3.7)	1.0	1.0	1.0	1.0
2 (3.8–7.3)	2.4 (1.4–4.0)[c]	1.7 (1.0–2.9)[b]	2.0 (1.2–3.5)[c]	1.6 (0.9–2.8)
3 (7.4–11.0)	5.3 (3.0–9.3)[d]	2.5 (1.4–4.5)[c]	3.6 (2.0–6.4)[d]	2.0 (1.1–3.8)[b]
4 (≥14.7)	9.6 (5.8–16.0)[d]	3.3 (1.9–5.9)[d]	6.1 (3.6–10.3)[d]	2.7 (1.5–4.9)[d]
BMI		1.2 (1.2–1.3)[d]		1.2 (1.2–1.3)[d]
WHR			1.1 (1.06–1.12)[d]	1.1 (1.04–1.09)[d]
P value for linear trend	<0.001	0.005	<0.001	0.031
Quartiles of SHBG (nmol/liter)				
1 (4.0–28.0)	1.0	1.0	1.0	1.0
2 (28.1–42.1)	0.4 (0.3–0.7)[d]	0.7 (0.5–1.1)	0.5 (0.4–0.8)[c]	0.8 (0.5–1.2)
3 (42.2–57.3)	0.3 (0.2–0.4)[d]	0.5 (0.3–0.8)[c]	0.4 (0.3–0.6)[d]	0.6 (0.4–1.0)[b]
4 (≥58.4)	0.06 (0.03–0.1)[d]	0.2 (0.08–0.3)[d]	0.1 (0.05–0.2)[d]	0.2 (0.1–0.4)[d]
BMI		1.2 (1.2–1.3)[d]		1.2 (1.2–1.3)[d]
WHR			1.1 (1.06–1.12)[d]	1.1 (1.03–1.09)[d]
P value for linear trend	<0.001	<0.001	<0.001	<0.001

Unadjusted models and models adjusted for BMI alone, WHR alone, and BMI and WHR together are presented. Likelihood ratio tests comparing each model with the previous model for each sex hormone and for SHBG yielded $P < 0.001$ for all comparisons. CI, Confidence interval.

[a] Defined as the highest HOMA-IR quartile; cutpoint = 2.1; mean for the highest quartile = 3.8 ± 1.9.

[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$.

However, unlike bioavailable testosterone, total testosterone levels were not associated with circulating insulin or with any measures of adiposity. The odds for IR were not increased even among individuals in the highest quartile of total testosterone. This discrepancy supports the hypothesis that stimulation of androgen production by insulin is a pathophysiology unique to PCOS (29) and that high bioavailable testosterone levels may be a marker for IR via a different mechanism in women unselected for PCOS.

Increasing both total and bioavailable levels of testosterone in obese postmenopausal women by administering exogenous androgens was recently shown to increase visceral adiposity as assessed by computer tomography (31). Visceral adiposity, in turn, is thought to impair hepatic insulin clearance and increase hepatic glucose output (32) and was shown to decrease insulin sensitivity among 380 Caucasian men and women [reviewed by Pratley and Weyer (33)]. In the present study, high levels of bioavailable testosterone were associated with increased odds of IR independent of WHR.

The increased odds of IR observed among the highest quartiles of bioavailable (non-SHBG bound) testosterone and estradiol in the present study may be a consequence of decreased SHBG. SHBG is negatively associated with hyperinsulinemia (34) and subsequent development of type 2 diabetes (35) among women and is accepted as a biological marker of hyperinsulinemic IR (29). A mechanism for this association has not been conclusively demonstrated (36), although *in vitro* studies show inhibition of hepatic SHBG production by insulin (37–39).

The present study is limited by the use of single hormone assays. The intraindividual variation of testosterone is low, however, and one measurement is thought to characterize an individual sufficiently well for epidemiological studies (40). In contrast, the intraindividual variation is high for estradiol,

suggesting that one measurement may not be sufficient (40). Nevertheless, expected associations of estradiol with estimates of total and central adiposity were found in this study. The low sensitivity of estrogen assays is a second potential limitation, but only 8% of this study population (66 of 866) had estradiol levels below the level of sensitivity, and exclusion of these women did not change the results. Imprecise measurements of adiposity could have resulted in residual confounding, but expected associations between BMI and WHR with measures of insulin resistance were observed, and BMI and WHR were not highly correlated with each other ($r = 0.42$). Finally, more precise measures of islet β-cell activity (such as the acute insulin response) and/or peripheral insulin sensitivity (glucose clamp technique) might enhance characterization of the sex hormone-IR relationship. Nevertheless, HOMA-IR has been shown to correlate strongly with euglycemic hyperinsulinemic clamp results ($R_s = 0.88$; Ref. 13).

In conclusion, the present study reports the expected association between bioavailable testosterone and IR. More surprising was the finding that higher total and bioavailable estradiol levels were also associated with IR and with stronger odds than those associated with androgen levels. This finding was independent of central adiposity as assessed by WHR. Thus, estrogen may be at least as important as testosterone in the pathway to IR in healthy, young postmenopausal women, with differences not entirely explained by body size. If these results are confirmed elsewhere, prospective studies will be necessary to determine the mechanism and the direction of the sex hormone-IR relationship.

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