Relationship Between Hormone Replacement Therapy Use With Body Fat Distribution and Insulin Sensitivity in Obese Postmenopausal Women

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The aim of this study was to compare the effect of hormone replacement therapy (HRT) on insulin resistance and central adiposity in obese postmenopausal women. Forty-five obese postmenopausal women (16 HRT users and 29 nonusers), with a mean age of 56.6 ± 5.3 years and duration of current, continuous HRT use of 4.7 ± 2.9 years, were included in the study. Subjects were studied using oral glucose tolerance tests, euglycemic clamping, dual photon x-ray absorptiometry, computed tomography, doubly labeled water, and treadmill testing. Insulin sensitivity, total fat, visceral fat, subcutaneous abdominal fat, thigh muscle attenuation, daily physical activity energy expenditure, peak oxygen consumption (Vo_2) were measured. HRT users had lower body weight (88.0 ± 11.0 *v* 98.2 ± 15.0 kg, $P = .05$), lower body mass index (33.1 ± 3.5 *v* 36.8 ± 5.2 kg/m^2, $P = .05$), lower fat mass (38.3 ± 7.3 *v* 44.1 ± 10 kg, $P = .05$), less visceral adipose tissue (157 ± 47 *v* 211 ± 81 cm^2; $P = .05$), and higher peak Vo_2 (21.1 ± 4.6 *v* 17.6 ± 2.2 mL/kg/min, $P = .001$) than nonusers. After adjustment for total fat, we noted a trend for decreased visceral adipose tissue in HRT users ($P = .09$). After adjustment for peak Vo_2, the decreased visceral adipose tissue persisted in HRT users ($P < .01$). Insulin sensitivity per killogram of lean body mass did not differ between HRT users (0.51 ± 0.22 mmol/kg/min) and nonusers (0.49 ± 0.22 mmol/kg/min). It was concluded that obese postmenopausal women using HRT have a more favorable body composition and fat distribution pattern than nonusers. Although visceral adipose tissue is decreased in HRT users, insulin sensitivity does not differ between HRT users and nonusers.

A CLUSTER OF metabolic abnormalities, including dyslipidemia, hypertension, increased central body fat, and glucose intolerance, is commonly referred to as the insulin resistance syndrome, leading to increased risk for cardiovascular disease.[1] It is known that central body fat and fasting insulin increase during the menopause transition, which could potentially lead to increased cardiovascular risk.[2,3] Hormone replacement therapy (HRT) given to postmenopausal women might improve these metabolic parameters, thus reducing the risk of cardiovascular disease.[4] Prospective trials of combined estrogen/progestin treatment using anthropometric methods or dual-energy x-ray absorptiometry (DXA) to measure central body fat found that HRT attenuated the increase in truncal body fat compared to placebo over a 1- to 3-year time span.[5-8] Although DXA allows for estimation of central fat by its measure of trunk fat, it does not distinguish between intra-abdominal and subcutaneous abdominal fat. Thus, the effects of HRT use on intra-abdominal fat and associated metabolic complications are unknown, particularly in obese women.

Studies of HRT and insulin sensitivity have been more controversial, with some studies finding improvement with HRT[9,10] and others finding no improvement or even reduced insulin sensitivity.[11-13] Discrepant results among investigators are partially attributable to indirect measurement of insulin sensitivity and small sample sizes. To our knowledge, no study has examined the relationship between current use of HRT, insulin sensitivity, and body composition using direct assessments of these parameters in obese postmenopausal women, a group at considerable risk for adverse metabolic and cardiovascular events. Furthermore, we considered other lifestyle variables such as prestudy weight fluctuation, aerobic fitness, and daily physical activity energy expenditure as possible confounders on body fat distribution and insulin sensitivity with HRT use in the present study. We hypothesized that current HRT use would be associated with lower total and visceral fat and improved insulin sensitivity.

SUBJECTS AND METHODS

Subjects

The study population consisted of 45 obese (35.7 ± 5.5 kg/m^2; mean ± SD) postmenopausal women between 50 and 71 years old (56.6 ± 5.3 years). The subjects were recruited by solicitation through the media between 1996 and 1998 for a study of genetics and weight loss. Individuals were included if their BMI was ≥27 kg/m^2, they had stopped menstruating for more than 1 year, and they had a follicle-stimulating hormone level of >30 mIU/mL. Participants also had to be sedentary (<2 times a week of exercise participation) nonsmokers and low to moderate alcohol consumers. All participants were apparently healthy and had no history or evidence on physical examination of (1) cardiovascular disease, peripheral vascular disease, or stroke; (2) diabetes; (3) moderate to severe hypertension (resting blood pressure >170/100 mm Hg); (4) body weight fluctuation of >5 kg in the previous 6 months; (5) thyroid or pituitary disease; or (6) medication that could affect cardiovascular function or metabolism. Sixteen of 45 women were taking HRT (mean ± SD duration of menopause, 7.5 ± 5.2 years; duration of HRT, 4.7 ± 2.9 years). HRT regimens included oral estradiol (n = 4), oral estradiol plus medroxyprogesterone acetate (n = 2), oral conjugated estrogens (n = 2), oral conjugated estrogens plus medroxyprogesterone acetate (n = 7), and vaginal conjugated estrogens (n = 1). All participants were asked to sign an informed consent document. The University of Vermont Institutional Review Board approved this study.

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Weight and Diet Stabilization Period

Subjects' weight was stabilized for 1 month before metabolic testing. This degree of experimental control is important because fluctuations in body weight influence insulin sensitivity.[14] Volunteers consulted with the dietitian on the General Clinical Research Center regarding energy and macro nutrient composition. Daily energy needs were then estimated from standardized equations developed in our laboratory.[15] During the weight-stabilization period, the diet consumed was approximately 30% of energy as fat, 58% as carbohydrate, and 12% as protein. Weight stability was verified by having subjects weighed twice per week at the Clinical Research Center. Macro nutrient composition was estimated by having subjects record their food intake for 3 days (2 weekdays and 1 weekend day). Three days before testing, dietary intake was provided and standardized for all the subjects (approximately 30% of energy as fat, 58% as carbohydrate, and 12% as protein) by the metabolic kitchen of the General Clinical Research Center.

Body Composition

Body weight was measured to the nearest 0.1 kg on a calibrated balance. Fat mass, lean body mass, and percentage of body fat were assessed using DXA (model DPX-L; Lunar Radiation Corp, Madison, WI) as previously described.[16] The subjects were asked to wear only a standard hospital gown and to maintain a supine position during the scan procedure.

Computed Tomography

Visceral adipose tissue and subcutaneous adipose tissue were measured by computed tomography (CT) as previously described (14,16,) using a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI). The subjects were examined in the supine position with both arms stretched above their heads. The position of the scan was established at the L4-L5 level using a scout image of the body. Visceral adipose tissue area was quantified by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. Adipose tissue was highlighted and computed using an attenuation range of −190 to −30 Hounsfield Units. The subcutaneous adipose tissue area was quantified by highlighting adipose tissue located between the skin and the external-most aspect of the abdominal muscle wall.

CT was also used to measure midthigh cross-sectional skeletal muscle and adipose tissue areas and muscle attenuation, the latter representing an estimate of muscle fat content.[17] Areas of skeletal muscle, adipose tissue, and muscle attenuation were calculated by delineating the regions of interest and then computing the surface areas using attenuation ranges of −190 to −30 Hounsfield units for adipose tissue and 0 to 100 Hounsfield units for skeletal muscle. Repeat measures of the different body fat distribution indices on 10 CT scans yielded a mean absolute difference of 1%. The radiation exposure of 200 millirems is well within the radiation exposure considered appropriate for normal subjects in the volunteer research environment, and all subjects gave informed consent according to local institutional review board regulations.

Measures of Energy Expenditure

Daily total energy expenditure (TEE) was determined from the doubly labeled water (DLW) over a 10-day period. During that period, subjects were asked to maintain their normal daily physical activity routines. The subjects were not participating in any structured exercise training program. Specific details about the DLW have been described extensively.[18,19]

Resting Metabolic Rate

Resting metabolic rate (RMR) was measured by indirect calorimetry using the ventilated hood technique[20] when the subjects awoke after a 12-hour overnight fast in the General Clinical Research Center. Respiratory gas analysis was performed using a Deltatrac metabolic cart (Sensormedics, Yorba Linda, CA). RMR (kcal/d) was calculated from the equation of Weir.[21] The test-retest correlation coefficient within 1 week has been shown to be 0.90 for RMR in our laboratory.

Daily Physical Activity Energy Expenditure

DLW in conjunction with indirect calorimetry was used to measure physical activity energy expenditure (PAEE). PAEE was calculated as the difference between TEE, RMR, and the thermic effect of a meal using the equation PAEE (kcal/d) = [TEE (kcal/d) × 0.9] − RMR (kcal/d), as previously described.[18,19] This approach assumes that the thermic effect of feeding is 10% of daily TEE in the elderly.[22] This measure was considered because differences in PAEE may help explain differences in visceral fat and insulin sensitivity.[23]

Peak Oxygen Consumption

Subjects performed a graded exercise test on treadmill to voluntary exhaustion to measure peak oxygen consumption (V_{O_2}) as previously described.[20] Standard 12-lead electrocardiograms were performed at the end of each 2-minute stage. Peak V_{O_2} (L/min) was considered the highest value obtained during the test. Expired gas was analyzed during the exercise protocol using a Sensormedics Horizon metabolic cart (Yorba Linda, CA). Data collection included V_{O_2} and respiratory equivalent ratio (CO_2 production/V_{O_2}).

Oral Glucose Tolerance Test

During an outpatient visit to the GCRC, a 2-hour 75-g oral glucose tolerance test (OGTT) was performed after 3 days of standardized diet (>250 g carbohydrate consumption) according to the guidelines of the National Diabetes Data Group. Insulin and glucose levels were measured at 0, 60, 90, and 120 minutes during the OGTT. The areas under the curve were determined by the trapezoid method.[24]

Euglycemic Hyperinsulinemic Clamping

Basal and insulin-stimulated glucose kinetics were measured by the euglycemic hyperinsulinemic clamp technique as described by DeFronzo et al[25] and implemented in our laboratory.[16,26] All subjects were tested upon awakening after a 12-hour overnight fast at the GCRC and 3 days of standardized meals. An intravenous catheter was placed in an antecubital vein at 6 AM for infusion of insulin, 20% dextrose, and [6,6-$^2H_2$]glucose (99% ^2H; Cambridge Isotope Laboratories, Andover, MA) tracer. A second catheter was placed retrograde in the contralateral hand for blood sampling. The hand was warmed in a box by a gentle stream of heated air (50° to 55°C) to produce arterialized venous blood. At 7 AM, a primed infusion of [6,6-$^2H_2$]glucose (4 mg/min) was begun and continued for 2 hours. Blood samples were taken before the start and during the second hour of the infusion for determination of plasma $^2H_2$-glucose enrichment. At 9 AM, the insulin infusion was begun and continued for 2 hours. Insulin is infused at a rate of 240 pmol/m^2/min to attain postprandial peripheral insulin levels and suppress hepatic glucose output. Blood glucose was monitored every 5 minutes during the insulin infusion, and euglycemia was maintained during clamping by infusing 20% dextrose at a variable rate. The duration of the insulin infusion was such that the rate of infused glucose reached a constant value by the second hour of the clamp. Blood samples were also taken during the last hour of clamping for determination of [6,6-$^2H_2$]glucose enrichment. To maintain a constant enrichment of [6,6-$^2H_2$]glucose tracer in blood during the clamp, [6,6-$^2H_2$]glucose was added to the 20% dextrose before the start of the study

to produce an enrichment of approximately 1 mol% excess (mpe) $^2H_2$-glucose in the dextrose. Aliquots of blood were placed in heparinized tubes and stored on ice until the plasma was prepared by centrifugation at 4°C, frozen, and stored at −60°C for later analysis.

Analytic Methods

For measurement of plasma [6,6-$^2H_2$]glucose enrichments, 0.1-mL aliquots of plasma were deproteinized with ice-cold (4°C) acetone, and the supernatants were decanted and placed into screw-cap vials, and the samples were evaporated to dryness under a gentle stream of dry nitrogen. After addition of 50 μL of 2% butyl boron dihydroxide (Sigma, St Louis, MO) in pyridine, the samples were allowed to sit for 24 hours at room temperature. Acetic anhydride (50 μL) was added just before measurement to complete formation of the butyl butylboronate glucose derivatization formation.

The butylboronate glucose derivatives were measured by gas chromatography–mass spectrometry using electron impact ionization (model 5971, Hewlett-Packard, Palo Alto, CA). The $[M\text{-}57]^+$ ions at m/z = 297 and 299 were monitored for unlabeled glucose and [6,6-$^2H_2$]glucose, respectively. The peak area ratios of 299/297 were determined by selected ion monitoring, as previously described.[27] From these ratios, the background corrected glucose enrichments in mpe were calculated.

Calculations

The purpose of euglycemic hyperinsulinemic clamping was to provide measurement of basal hepatic glucose output or appearance (Ra) and of hepatic glucose output during clamping. The rate of glucose output (HGO) was calculated from the mean [6,6-$^2H_2$]glucose enrichment in plasma during the basal state: $HGO = I\ (E_i / E_p - 1)$, where I is the rate of [6,6-$^2H_2$]glucose infusion (mg/min), E_i is the enrichment of the tracer enrichment in mpe, and E_p is the mean enrichment (mpe) of [6,6-$^2H_2$]glucose in plasma in the basal state.

During euglycemic hyperinsulinemic clamping, total glucose disposal (TGD) was also calculated from the plasma $^2H_2$-glucose enrichment taken from blood samples during the last 30 minutes of the insulin infusion: $TGD = (IE_i + ME_m)/E_p - I$, where M is the rate of exogenous dextrose infusion (mg/min) and E_m is the enrichment of [6,6-$^2H_2$]glucose in the infused dextrose (mpe).

The hepatic glucose output during clamping (HGO_{cl}) was taken as the difference between TGD measured using the $^2H_2$-glucose and the mean rate of dextrose infusion during the last 30 minutes of the insulin infusion (M): $HGO_{cl} = TGD - M$.

Analyses of the clamp procedure indicated that a coefficient of variation of 9% was obtained for plasma glucose levels during clamping for the entire cohort.

Biochemical Analyses

Plasma glucose concentrations were determined using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin levels were determined by use of a double-antibody radioimmunoassay (RIA; Diagnostic Products Corp, Los Angeles, CA).

Statistical Analyses

Data are presented as means ± 1 SD. Pearson product-moment correlations were used to determine the relationship between dependent and independent variables. Because total adipose tissue and peak V_{O_2} may influence the association between visceral adipose tissue and glucose disposal, the relationship between these variables was examined after statistical adjustment for total fat mass and peak V_{O_2} using partial correlation analyses. Analysis of covariance was used to adjust means for potential confounders. Unpaired t tests were used to examine differences between groups. Log transformation was used to normalize the distribution for variables of interest that had an abnormal distribution (age, BMI, visceral adipose tissue, insulin at fasting state and during OGTT, basal hepatic glucose output, hepatic glucose output during clamping, total glucose disposal, and total glucose disposal expressed per killigram of lean body mass). A P level less than .05 was considered significant.

RESULTS

The body composition and body fat distribution of study volunteers are shown in Table 1. No differences in age or activity level were measured by DLW between current users and nonusers of HRT. However, users of HRT had a slightly higher peak V_{O_2} ($P < .001$), a lower body weight ($P < .05$), and a lower BMI ($P < .05$) than nonusers. HRT users had significantly less fat mass than nonusers (5.8 kg, $P < .05$).

Table 1. Body Composition and Body Fat Distribution in Users and Nonusers of HRT

	Nonusers (n = 29)	Users (n = 16)	*P*
Age (yr)	57.4 ± 5.8	54.2 ± 3.6	NS
Body weight (kg)	98.2 ± 15.0	88.0 ± 11.0	<.05
Body mass index (kg/m^2)	36.8 ± 5.2	33.1 ± 3.5	<.05
Absolute peak V_{O_2} (L/min)	1.73 ± 0.31	1.85 ± 0.47	NS
Peak V_{O_2} (mL/kg/min)	17.6 ± 2.2	21.1 ± 4.6	<.001
PAEE (kcal/d)	1149 ± 260	1252 ± 426	NS
DXA measures			
Body fat (%)	48.1 ± 4.3	45.7 ± 4.7	NS
Fat mass (kg)	44.1 ± 10.0	38.3 ± 7.3	<.05
Lean body mass (kg)	47.5 ± 6.3	44.8 ± 5.3	NS
CT scan area measures			
Abdomen level (L4-L5)			
Subcutaneous adipose tissue (cm^2)	503 ± 118	489 ± 87	NS
Visceral adipose tissue (cm^2)	211 ± 81	157 ± 47	<.05
VAT adjusted for total fat mass	205 ± 70	167 ± 68	<.09
Midthigh level			
Mean subcutaneous adipose tissue (cm^2)	204 ± 60	175 ± 56	NS
Mean muscle area (cm^2)	111 ± 17	111 ± 16	NS
Mean attenuation of muscle (Hounsfield U)	43.0 ± 4.2	44.2 ± 3.1	NS

However, percent body fat and lean body mass did not significantly differ between groups.

Differences in body fat distribution between groups showed that current users of HRT had approximately 54 cm^2 less visceral adipose tissue than nonusers ($P < .05$). After adjustment for total fat mass, HRT users tended to have less visceral adipose tissue than nonusers. When visceral fat was expressed as a percentage of total abdominal fat at L4-L5, visceral fat was lower in users (0.24) than in nonusers (0.30). To further support this finding, we also matched 15 HRT users and 15 nonusers for total fat mass and found a trend toward less visceral adipose tissue in the HRT users ($P = .1$, data not shown). After adjustment for peak Vo_2, the amount of visceral adipose tissue remained lower in HRT users. Subcutaneous adipose tissue did not differ between nonusers and users of HRT. At the midthigh, mean subcutaneous adipose tissue, mean attenuation of muscle by adipose, and mean muscle area were not different between nonusers and users of HRT.

Table 2 shows that insulin resistance, as measured by the OGTT, and glucose sensitivity did not differ between HRT users and nonusers. We found no difference in fasting insulin or glucose levels or in the areas under the curve between groups. Furthermore, basal hepatic glucose output, hepatic glucose during clamping, or total glucose disposal per killigram of lean body mass did not differ between groups.

Table 3 shows correlations between total glucose disposal per lean body mass and peak Vo_2, body weight, BMI, fat mass, visceral fat, and thigh fat. Although visceral adipose tissue was significantly correlated to glucose disposal per lean body mass in both current users and nonusers of HRT, there were no significant differences between groups. When adjusted for total fat, correlations between visceral adipose tissue and total glucose disposal per lean body mass remained insignificant between groups (data not shown). Similarly, no significant differences were noted between HRT users and nonusers in any other correlation between glucose disposal and body fat or fitness measurement.

Table 2. Responses During OGTT and Clamping for Users and Nonusers of HRT

	Nonusers (n = 29)	Users (n = 16)	*P*
OGTT			
Fasting insulin (pmol/L)	143 ± 96	100 ± 52	NS
Fasting glucose (mmol/L)	5.2 ± 0.7	4.9 ± 0.6	NS
Insulin area (pmol/L × 10^{-3})	96.1 ± 67.8	79.4 ± 47.5	NS
Glucose area (mmol/L × 10^{-3})	0.87 ± 0.19	0.90 ± 0.18	NS
Clamping			
Basal hepatic glucose output (mmol/min)	9.7 ± 1.8	8.9 ± 1.0	NS
Hepatic glucose output during clamp (mmol/min)	3.2 ± 3.0	2.2 ± 1.4	NS
Exogenous glucose infusion (mmol/min)	19.9 ± 8.0	20.6 ± 8.2	NS
Total glucose disposal (mmol/min)	23.1 ± 9.5	22.8 ± 8.8	NS
Total glucose disposal (mmol/kg LBM/min)	0.49 ± 0.22	0.51 ± 0.22	NS

Abbreviation: LBM, lean body mass.

Table 3. Correlations Between Independent Variables and Insulin Sensitivity in Users and Nonusers of HRT

	Total Glucose Disposal/LBM (mg/kg/min)		Different Groups
	Nonusers	Users	
Age (yr)	−0.22	−0.02	NS
Peak Vo_2 (mL/kg/min)	−0.26	−0.36	NS
Body weight (kg)	−0.24	−0.10	NS
Body mass index (kg/m^2)	−0.34	0.13	NS
DXA measures			
Fat mass (kg)	−0.07	0.23	NS
Lean body mass (kg)	−0.26	−0.46	NS
CT scan measures			
Abdomen level (L4-L5)			
Subcutaneous adipose tissue (cm^2)	0.08	0.08	NS
Visceral adipose tissue (cm^2)	−0.53*	−0.52†	NS
Mid-thigh level			
Mean subcutaneous adipose tissue (cm^2)	0.13	0.32	NS
Mean attenuation of muscle (Hounsfield U)	0.02	0.26	NS

* $P < .005$.

† $P < .05$.

DISCUSSION

To our knowledge, this is the first study to examine whether current use of HRT is associated with differences in visceral fat and insulin sensitivity as measured from direct assessments of these parameters. Prior studies have been limited in their use of proxy measures of fat (anthropometric measures) and insulin sensitivity (OGTT only). Other studies have used DXA to measure trunk fat,[5,28] which cannot distinguish between visceral and subcutaneous abdominal fat.

We originally hypothesized that HRT use would be associated with lower visceral and total adiposity and improved insulin sensitivity. Our findings partially support this hypothesis. That is, we found decreased visceral adipose tissue compared with total abdominal adipose tissue in obese postmenopausal women using HRT compared with those not using HRT. Moreover, a trend was noted in HRT users having less visceral adipose tissue than nonusers even when adjusted for total fat mass. This suggests that HRT use may selectively reduce visceral fat. Visceral adipose tissue remained less in HRT users than in nonusers after adjustment for peak Vo_2 also ($P < .01$), suggesting that fitness differences in these women were not confounders. The fact that subcutaneous fat did not differ between users and nonusers emphasizes the importance of using CT scanning or magnetic resonance imaging to measure the intra-abdominal fat compartment in studies of this type.

Our findings of reduced central fat in current users of HRT are consistent with those of Haarbo et al[5] and Kristensen et al,[28] who used DXA to measure trunk fat, and that of Reubinoff et al[6] and Perrone et al,[29] who reported lower waist-to-hip ratios, a surrogate for visceral index, in women using HRT. However, no CT scan measurements of visceral fat were obtained in these studies. Our findings contrast with those of Aloia et al,[30] who

found no meaningful effect of HRT in decreasing trunk fat measurements obtained by DXA.

Adipose tissue itself is a source of endogenous estrogen. Because the non-users had more body fat, we would expect their endogenous estrogen levels to be higher than HRT users. This could reduce the differences between groups.

Adipose tissue in the midthigh has been shown to influence insulin-stimulated glucose disposal as measured by euglycemic hyperinsulinemic clamping.[17] Although these authors found that muscle attenuation at the thigh, which is a proxy indicator of muscle fat accumulation, correlated with insulin-stimulated glucose disposal, we found no difference in thigh muscle attenuation between users and nonusers of HRT. Furthermore, attenuation of muscle by fat in the thigh or subcutaneous thigh fat did not relate to total glucose disposal in users or nonusers of HRT in our study. Thus, our results do not support a meaningful effect of muscle fat accumulation as a correlate of insulin sensitivity.

Peak V_{O_2}, reflecting aerobic fitness level, differed between users and nonusers. This finding is consistent with other studies showing that women who choose to take HRT maintain a healthier lifestyle than those who choose not to take it.[31] Despite the differences in peak V_{O_2}, no differences in PAEE were noted between groups. Peak V_{O_2} or PAEE was not found to be a strong predictor of insulin sensitivity in our population. It is possible that the relatively homogenous nature of our cohort (all were obese and inactive) limits our ability to examine the relationship between physical activity–related parameters and insulin sensitivity.

To our knowledge, only 2 studies have used euglycemic clamping to measure insulin sensitivity in postmenopausal women taking HRT.[12,13] Our finding of no difference in insulin sensitivity between obese current users and nonusers of HRT despite differences in intra-abdominal fat and total abdominal fat was unexpected. However, it agrees with those of others who found that the route of estrogen administration did not influence insulin sensitivity in postmenopausal women[12] and that women who underwent surgical menopause had no detectable improvement in insulin sensitivity with transdermal estradiol with or without progestin.[13] However, both of these studies were performed in nonobese postmenopausal women.

We would suggest that the discordance between lower levels of visceral adipose tissue and improved insulin sensitivity in our study probably relates to the nature of the cohort studied. All women were obese, and both groups had high levels of intra-abdominal fat. It is possible that once a certain threshold of total and intra-abdominal fat is obtained (>130 cm^2), the ability to discriminate differences in insulin sensitivity within an obese population is reduced.[32,33] Another possible explanation is that insulin resistance takes more time to develop after intra-abdominal fat is deposited, which may not yet be detectable in our study. Furthermore, the range of insulin sensitivity of our volunteers was small.

Several features of our experimental approach enhance the credibility of our findings. We performed direct assessments of our outcome variables (insulin sensitivity, visceral fat, and PAEE) with criterion measurements. Second, careful attention was paid to stabilizing body weight and dietary intake before metabolic assessments were performed. Third, we also considered other potential confounders in our experimental design (fitness and physical activity) that may influence insulin sensitivity. Nonetheless, the cross-sectional nature of our design precludes any notion of cause and effect. Only randomized trials can demonstrate the efficacy of HRT to reduce visceral fat and improve insulin sensitivity.

We conclude that obese postmenopausal women who use HRT have a greater peak V_{O_2}, less visceral adipose tissue compared with total adipose tissue, but no difference in insulin sensitivity compared to nonusers. Further prospective, randomized trials in both obese and nonobese populations of postmenopausal women are needed to clarify the effects of HRT on parameters of the insulin resistance syndrome.

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