Hormone Replacement Therapy, Insulin Sensitivity, and Abdominal Obesity in Postmenopausal Women

Alice S. Ryan, PhD
Barbara J. Nicklas, PhD
Dora M. Berman, PhD

OBJECTIVE — The purpose of this study was to determine whether insulin sensitivity differs between postmenopausal women taking estradiol, women on estrogen plus progesterone hormone replacement therapy (HRT), and women not on HRT and whether differences are explained by the differences in total and/or abdominal adiposity and fat deposition in the muscle.

RESEARCH DESIGN AND METHODS — We studied 28 obese, sedentary postmenopausal Caucasian women. Women taking oral estrogen (n = 6) were matched for age (57 ± 3 vs. 58 ± 2 years), weight (87.9 ± 6.0 vs. 83.0 ± 3.9 kg), and BMI (33.9 ± 1.7 vs. 33.9 ± 1.9 kg/m²) with women not on HRT (n = 6). Eight women taking oral estrogen plus progesterone were matched with eight different women not on HRT for age (59 ± 2 vs. 60 ± 2 years), weight (82.8 ± 3.7 vs. 83.7 ± 4.1 kg), and BMI (30.7 ± 1.0 vs. 29.9 ± 1.3 kg/m²).

RESULTS — VO₂max (maximal aerobic capacity), percentage of fat, total body fat mass, and fat-free mass (FFM) were similar between groups. Visceral fat, subcutaneous abdominal fat, sagittal diameter, and mid-thigh low-density lean tissue (intramuscular fat) did not differ by hormone status. Basal carbohydrate and fat utilization was not different among groups. Fasting plasma glucose and insulin did not differ by hormone use. Glucose utilization (M) was measured during the last 30 min of a 3-h hyperinsulinemic-euglycemic clamp (40 mU · m² · min⁻¹). Postmenopausal women taking oral estrogen had a 31% lower M than women not on HRT (42.7 ± 4.0 vs. 61.7 ± 4.7 µmol · kg⁻¹ · FFM⁻¹ · min⁻¹, P < 0.05). M was 26% lower in women taking estrogen plus progesterone (44.0 ± 3.5 vs. 59.7 ± 6.2 µmol · kg⁻¹ · FFM⁻¹ · min⁻¹, P < 0.05) than women not on HRT. M/I, the amount of glucose metabolized per unit of plasma insulin (I), an index of insulin sensitivity, was 36% lower in women taking estrogen compared with matched women not on HRT (P < 0.05) and 28% lower in women taking estrogen plus progesterone compared with matched women not on HRT (P < 0.05).

CONCLUSIONS — Postmenopausal women taking oral estrogen or those taking a combination of estrogen and HRT are more insulin-resistant than women not on HRT, even when women are of comparable total and abdominal adiposity.

Diabetes Care 25:127–133, 2002

Postmenopausal estrogen therapy and estrogen plus progesterone hormone replacement therapy (HRT) alleviate symptoms of menopause and attenuate bone loss (1). Moreover, several observational studies suggest that use of estrogen replacement therapy decreases the risk of coronary heart disease (2–4) and lowers overall mortality rates (5,6).

The menopause transition, as well as the early postmenopausal period, is associated with an increase in total and central obesity (7–9). Increased visceral fat is associated with insulin resistance (10), and this preferential storage of abdominal fat may contribute to cardiovascular disease and diabetes in postmenopausal women. Estrogen and HRT may improve fat distribution in postmenopausal women by preventing the increase in central body fat (7,11–13). However, the evidence concerning the effects of HRT on glucose homeostasis is controversial. Estrogen replacement therapy has been reported to have no effect on insulin sensitivity in postmenopausal women (14–16) and to improve carbohydrate metabolism in individuals with type 2 diabetes (17,18). In contrast, nondiabetic women taking estrogen alone were more insulin-resistant than women not on HRT or women taking estrogen and HRT (19). Differences in the study population, type and route of administration of hormone therapy, and method of measuring insulin sensitivity may explain the disparate results of estrogen replacement on glucose metabolism.

Potential variations in physical fitness and adiposity, specifically visceral fat and fat deposition in the muscle, which are associated with insulin resistance (20–22), could also contribute to differences in insulin sensitivity between women on and not on HRT. It is unknown whether there are differences in fat deposition in the muscle between women who take estrogen, estrogen plus progesterone, and those not on HRT. Nevertheless, a comparison of insulin sensitivity and concomitant characterization of total and visceral obesity and mid-thigh low-density lean tissue (a marker of intra-muscular fat) in

From the Department of Medicine, Division of Gerontology at the University of Maryland School of Medicine, and the Baltimore Geriatric Research, Education and Clinical Center (GRECC), VA Maryland Health Care System, Baltimore, Maryland.

Address correspondence and reprint requests to Alice S. Ryan, PhD, Division of Gerontology, BT/18/GR, 10 N. Greene St., Baltimore Veterans Affairs Medical Center, Baltimore, MD 21201. E-mail: aryan@grecc.umaryland.edu.

Received for publication 6 June 2001 and accepted in revised form 12 September 2001.

Abbreviations: CT, computed tomography; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; HRT, hormone replacement therapy; HU, Hounsfield unit; I, plasma insulin; IGT, impaired glucose tolerance; ITT, insulin tolerance test; IVGTT, intravenous glucose tolerance test; M, glucose utilization; OGTT, oral glucose tolerance test; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
women taking estrogen, taking estrogen plus progesterone, and not taking hormone therapy has not been conducted.

We tested the hypothesis that estrogen would improve insulin sensitivity due to a reduction in abdominal fat accumulation. Therefore, the purpose of this study was to determine whether insulin sensitivity differs between overweight and obese, sedentary postmenopausal Caucasian women taking estrogen and estrogen plus progesterone versus Caucasian women not on HRT matched for age, body weight, and BMI and whether differences are explained by total and/or abdominal adiposity and intramuscular thigh fat.

RESEARCH DESIGN AND METHODS — All subjects were healthy, overweight, and obese (BMI > 25 kg/m²) Caucasian women aged 50–70 years and were at least 1 year postmenopause. Only women who were weight stable (< 2.0 kg weight change in past year) and sedentary (< 20 min of aerobic exercise two times per week) were recruited. Subjects were screened by medical history questionnaire, physical examination, fasting blood profile, and a graded exercise treadmill test in an attempt to exclude those with cardiovascular disease. The women underwent a 2-h 75-g oral glucose tolerance test (OGTT) (23). Blood samples were collected at baseline and at the 2-h period for measurement of plasma glucose. All subjects were nonsmokers, had no evidence of diabetes, and had no evidence of cancer, liver, renal or hematological disease, or other medical disorders. A total of 43 women met all study criteria and were enrolled into the study. Six women were using the conjugated estrogen Premarin 0.625 mg g.d. (n = 4), estradiol 0.5 mg g.d. (n = 1), or estradiol 1 mg g.d. (n = 1), and eight women were using combined oral estrogen and HRT (Prempro 0.625 mg/2.5 mg q.d.; conjugated estrogen plus medroxyprogesterone acetate). All women had been taking estrogen plus progesterone (Prempro 0.625 mg/2.5 mg q.d.; conjugated estrogen plus medroxyprogesterone acetate). All women had been taking estrogen plus medroxyprogesterone (Premarin 0.625 mg g.d. (estradiol 0.5 mg g.d. (n = 4), or estradiol 1 mg g.d. (n = 1), and eight women were using combined oral estrogen and HRT (Prempro 0.625 mg/2.5 mg q.d.; conjugated estrogen plus medroxyprogesterone acetate). All women had been taking estrogen plus progesterone versus Caucasian women not on HRT matched for age, body weight, and BMI and whether differences are explained by total and/or abdominal adiposity and intramuscular thigh fat.

Maximal oxygen uptake
Maximal oxygen uptake (V̇O₂max) was measured using a continuous treadmill test protocol as described previously (24). Briefly, speed was kept constant while the grade was increased from 0 to 4% at 2 min and then increased 2% every minute after the third minute until the woman was unable to continue. Validation for attainment of V̇O₂max included meeting two of the following three criteria: 1) a plateau in oxygen uptake with an increased workload as evidenced by a difference in oxygen uptake of < 2 ml · kg⁻¹ · min⁻¹, 2) a respiratory exchange ratio > 1.10, and 3) a maximal heart rate within 10 beats/min of the age-predicted maximal value.

Body composition
Anthropometry. Height (cm) and weight (kg) were measured to calculate BMI as weight (kg)/height (m²). Waist circumference, measured at the narrowest point superior to the hip, was divided by the circumference of the hip, measured at its greatest gluteal protuberance, to obtain waist-to-hip ratio (WHR).

Dual-energy X-ray absorptiometry.
Fat mass, lean tissue mass, and bone mineral content were determined by dual-energy X-ray absorptiometry (DXA) (model DPX-L; Lunar Radiation, Madison, WI). Fat-free mass (FFM) is reported as lean tissue plus bone mineral content. All DXA scans were analyzed using the LUNAR Radiation version 1.3z, model DPX-L extended analysis program for body composition analyses.

Computed tomography. To quantify visceral and abdominal subcutaneous fat areas, a computed tomography (CT) scan of the abdomen was performed using a PQ 6000 Scanner (Marconi Medical Systems, Cleveland, OH). A single 5-mm scan was taken at the L₅–L₆ region while the subject was supine with her arms stretched above her head. A fat tissue-highlighting technique was used to quantify the relative proportions of visceral adipose tissue and subcutaneous adipose tissue areas. Sagittal diameter was determined based on the images at the level of the umbilicus and the fourth lumbar intervertebral disk. CT data are expressed as cross-sectional area of tissue (cm²) with the Hounsfield units (HU) for adipose tissue as –190 to –30. A second scan performed at the level of the mid-thigh was used to quantify muscle area (HU 30–80), total fat area of the thigh (HU –190 to –30), and low-density lean tissue (HU 0–29) of both the right and left legs, as described previously (25). One CT scan was missing from the group of women taking estrogen plus progesterone; therefore, data from one woman not on HRT was not used in the analysis.

Metabolic testing
To control nutrient intake before the clamp studies, all subjects were provided with a eucaloric diet for 2 days before testing by a registered dietitian. The composition of this diet was 50–55% carbohydrate, 15–20% protein, ≤ 30% fat, and 300–400 mg cholesterol per day and a polyunsaturated to saturated fat ratio of 0.6–0.8. The diet was composed of at least 150 g carbohydrate/day for the 2 days before testing (26). The number of calories given to each woman was estimated from the 7-day food records, and estimates of energy expenditure were based on the Harris-Benedict equation (27). All testing was performed in the morning after a 12-h overnight fast. All subjects were weight stabilized (< 1 kg) for at least 2 weeks before metabolic testing.

Hyperinsulinemic-euglycemic clamps.
Peripheral tissue sensitivity to exogenous insulin was measured using the hyperinsulinemic-euglycemic clamp technique (28). Briefly, an intravenous catheter was inserted by percutaneous venipuncture for the infusion of glucose and insulin. A second catheter was inserted in a retrograde fashion into a dorsal hand or wrist vein, and the hand was enclosed in a grounded, insulated chamber warmed to 70°C to “arterialize” (29) the blood obtained for all samples. For the assessment of basal glucose and insulin levels, three arterialized blood samples were collected at 10-min intervals. Blood samples were obtained every 5 and 10 min thereafter for the determination of plasma glucose and insulin levels. A 10-min priming and continuous infusion of insulin (240 pmol •
Table 1—Physical characteristics of overweight and obese postmenopausal women by HRT status

<table>
<thead>
<tr>
<th></th>
<th>Estrogen (n = 6)</th>
<th>vs. No HRT1 (n = 6)</th>
<th>Estrogen plus progesterone (n = 8)</th>
<th>vs. No HRT2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57 ± 3</td>
<td>58 ± 2</td>
<td>59 ± 2</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.9 ± 6.0</td>
<td>83.0 ± 3.9</td>
<td>82.8 ± 3.7</td>
<td>83.7 ± 4.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.9 ± 1.7</td>
<td>33.9 ± 1.9</td>
<td>30.7 ± 1.0</td>
<td>29.9 ± 1.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>98.6 ± 4.3</td>
<td>95.2 ± 5.2</td>
<td>92.9 ± 3.0</td>
<td>93.0 ± 3.2</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>119.9 ± 3.8</td>
<td>116.4 ± 2.9</td>
<td>111.2 ± 2.9</td>
<td>114.2 ± 3.4</td>
</tr>
<tr>
<td>WHR</td>
<td>0.83 ± 0.03</td>
<td>0.82 ± 0.03</td>
<td>0.84 ± 0.03</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>VO2max (ml·kg·min⁻¹)</td>
<td>19.3 ± 1.1</td>
<td>20.7 ± 1.7</td>
<td>18.5 ± 1.5</td>
<td>20.7 ± 0.6</td>
</tr>
</tbody>
</table>

Data are means ± SEM.

m⁻²·min⁻¹) (Humulin; Eli Lilly, Indianapolis, IN) was performed for 180 min. This resulted in a square wave of hyperinsulinemia at a level of 486 ± 14 pmol/l among the women. A 20% dextrose solution was used, which was measured as 18%.

The mean plasma glucose level during 10–180 min of the euglycemic clamp was computed for each individual study and expressed as a percentage of the desired goal. The plasma glucose levels during each clamp period averaged 5.18 ± 0.17 vs. 5.47 ± 0.15 mmol/l for estrogen versus no HRT1 and 5.19 ± 0.17 vs. 5.22 ± 0.16 mmol/l for estrogen plus progesterone versus no HRT2. This was 97.5 ± 0.3 vs. 97.3 ± 0.2% of the desired goal with a coefficient of variation of 4.9 ± 0.9 vs. 5.1 ± 0.5% for estrogen versus no HRT1. For the estrogen plus progesterone versus no HRT2 group, this was 97.8 ± 0.2 vs. 97.7 ± 0.3% of the desired goal with a coefficient of variation of 4.3 ± 0.4 vs. 4.7 ± 0.3%. Plasma insulin concentrations during 150–180 min of the hyperinsulinemic-euglycemic clamps were comparable between groups (estrogen versus no HRT1, 350 ± 37 vs. 494 ± 25 pmol/l; estrogen plus progesterone versus no HRT2, 476 ± 32 vs. 472 ± 19 pmol/l; P = NS).

Indirect calorimetry. To quantitate carbohydrate oxidation, continuous indirect calorimetry was performed before the start of the glucose infusion and during the last 30 min of the insulin infusion by the open-circuit dilution technique using a SensorMedics DeltaTrac cart (Torba Linda, CA). Rates of glucose oxidation were calculated from measurements of carbon dioxide production and oxygen consumption using established equations (30) with correction for protein oxidation determined from 24-h urinary urea nitrogen. Nonoxidative glucose metabolism was calculated as the difference between total glucose uptake and glucose oxidation.

Analysis of blood samples. Blood samples were collected in heparinized syringes and placed in prechilled test tubes containing 1.5 mg EDTA/ml blood in a total volume that was 4% of the sample volume. The blood samples were centrifuged at 4°C, and a 1-ml aliquot of plasma was rapidly frozen (80°C) for subsequent hormone analysis. All determinations were performed in duplicate. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Immunoreactive insulin was determined by an insulin-specific double-antibody system as described previously (31) using human insulin standards and tracer. The antiserum was raised against highly purified human insulin and does not cross-react with human proinsulin (<0.1%) (Linco, St. Louis, MO). The lower limit of detection of this assay in our laboratory is 12 pmol/l. Intra- and interassay coefficients of variation of pooled control sera averaged 5 and 9%, respectively.

Statistical analyses. The mean concentration of glucose and insulin was calculated for each sample time point for the clamp. The trapezoidal rule was used to calculate the integrated response over 30-min intervals from 30–180 min for each subject. The integrated response was divided by its time interval to compute mean concentrations. Glucose utilization (M) for 30-min intervals was calculated from the amount of glucose infused after correction for glucose equivalent space (glucose space correction). Insulin sensitivity was expressed as M/I, which represents the amount of glucose metabolized per unit of plasma insulin (1), and was calculated by dividing the glucose used by the insulin concentration during the last 30 min of the clamp for each subject. Statistical significance between groups (estrogen versus no HRT1) and (HRT versus no HRT2) was determined by Student’s t tests. All data were analyzed using SPSS statistical software (SPSS, Chicago, IL). Data are expressed as means ± SEM, and significance was set at the P < 0.05 level.

RESULTS — The physical characteristics of the women are presented in Table 1. Age, body weight, BMI, waist and hip circumferences, WHR, and VO2max (ml·kg⁻¹·min⁻¹) were similar between women taking estrogen versus women in the no-HRT1 group and between women taking estrogen plus progesterone versus women in the no-HRT2 group. The comparison among matched groups showed no differences in percentage of fat, fat mass, FFM, visceral adipose tissue, subcutaneous abdominal adipose tissue area, sagittal diameter, mid-thigh muscle area, mid-thigh subcutaneous fat, or mid-thigh low-density lean tissue area (Table 2).

As a result of the screening OGTT, two women (33%) in the estrogen group and two women (33%) not on HRT (HRT1 group) were found to have impaired glucose tolerance (IGT). The same number of women were found to have IGT in the estrogen plus progesterone group (n = 2, 25%) and in the group of women not on HRT (HRT2 group; n = 2, 25%). Fasting plasma glucose and insulin concentrations did not differ by hormone status (Table 3). Glucose utilization during the last 30 min (150–180 min) of the clamp was 31% lower in women taking estrogen than in women not on HRT (no HRT1) (42.7 ± 4.0 vs. 61.7 ± 4.7 μmol·
**HRT and insulin resistance**

Table 2—Total and regional body composition by DXA and CT of overweight and obese postmenopausal women by HRT status

<table>
<thead>
<tr>
<th></th>
<th>Estrogen (n = 6) vs. No HRT1 (n = 6)</th>
<th>Estrogen plus progesterone (n = 8) vs. No HRT2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent body fat</td>
<td>48.8 ± 1.7</td>
<td>46.6 ± 1.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>42.3 ± 3.3</td>
<td>38.4 ± 2.4</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>44.2 ± 2.7</td>
<td>43.2 ± 1.6</td>
</tr>
<tr>
<td>Abdominal VAT area (cm²)</td>
<td>167.5 ± 20.6</td>
<td>130.0 ± 15.0</td>
</tr>
<tr>
<td>Subcutaneous abdominal adipose area (cm²)</td>
<td>530.4 ± 39.8</td>
<td>411.3 ± 30.8</td>
</tr>
<tr>
<td>Sagittal diameter (mm)</td>
<td>27.0 ± 1.1</td>
<td>24.4 ± 0.7</td>
</tr>
<tr>
<td>Mid-thigh Fat (cm²)</td>
<td>184.1 ± 15.3</td>
<td>151.0 ± 18.2</td>
</tr>
<tr>
<td>Muscle (cm²)</td>
<td>85.8 ± 9.3</td>
<td>69.2 ± 6.2</td>
</tr>
<tr>
<td>Low-density lean tissue (cm²)</td>
<td>15.5 ± 3.5</td>
<td>14.3 ± 3.0</td>
</tr>
</tbody>
</table>

Data are means ± SEM. VAT, visceral adipose tissue.

*kgFFM⁻¹·min⁻¹, P < 0.05* (Fig. 1). M was 26% lower in the women taking estrogen plus progesterone than the women in the no-HRT2 group (44.0 ± 3.5 vs. 59.7 ± 6.2 μmol·kg⁻¹·min⁻¹, P < 0.05). M/I, an index of insulin sensitivity, was 36% lower in the women taking estrogen than the women not on HRT (P < 0.05) and 28% lower in women taking estrogen plus progesterone than the matched women not on HRT (P < 0.05). Nonoxidative glucose disposal was 46% higher in the women in the no-HRT1 group than in the estrogen group (P < 0.01) and 28% higher in women not on HRT (no HRT2) compared with the estrogen plus progesterone group, but this was not statistically significant. Basal glucose oxidation rates did not differ between the group comparisons, but insulin-stimulated glucose oxidation was 39% higher in women not on HRT (no HRT1) than the comparison group of women taking estrogen (P < 0.05) but did not differ between women not on HRT (no HRT2) compared with the estrogen plus progesterone group.

**CONCLUSIONS** — The results of the current study indicate that use of estrogen in postmenopausal women contributes to the variability in insulin sensitivity observed in overweight and obese, sedentary women. Specifically, women taking oral estrogen and estrogen plus progesterone have lower glucose utilization and insulin sensitivity than women who are not on HRT matched for age, weight, and obesity.

Similar to our findings, additional reports conclude that estrogen therapy may, in fact, worsen glucose homeostasis (18,34–36). Randomized trials of postmenopausal women indicate that 6–18 months of HRT increase fasting insulin levels (32) and decrease insulin sensitivity using either the intravenous glucose tolerance test (IVGTT) (33) or the insulin tolerance test (ITT) (34). Our results also agree with a recent cross-sectional study (19) in which postmenopausal women taking estrogen only had lower insulin sensitivity compared with women not on HRT and women taking estrogen plus progesterone. In fact, the women taking estrogen had 31% lower glucose utilization and a 35% lower insulin sensitivity using M/I compared with the matched women not on HRT. This is very similar to the 29% lower insulin sensitivity index by IVGTT in sedentary women on HRT compared with those not on HRT previously reported (19).

In contrast, several studies suggest that estrogen replacement therapy improves glucose homeostasis (18,34–36). Estrogen-treated women had significantly lower fasting insulin levels than women not taking estrogen (19), even after adjustment for age and WHR (35). Glucose utilization by the hyperinsulinemic-euglycemic clamp had a tendency to increase, and HbA1c significantly decreased after 3 months of oral estradiol therapy in postmenopausal women with type 2 dia-

Table 3—Effects of HRT on insulin sensitivity in overweight and obese postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>150–180 min of clamp</th>
<th>Nonoxidative glucose disposal</th>
<th>Oxidative glucose disposal</th>
<th>M/I (μmol·kg⁻¹·min⁻¹)/(pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma glucose (mmol/l)</td>
<td>5.3 ± 0.1</td>
<td>19.4 ± 2.5*</td>
<td>15.9 ± 2.4*</td>
<td>0.042 ± 0.005*</td>
</tr>
<tr>
<td></td>
<td>Plasma insulin (pmol/l)</td>
<td>66 ± 10</td>
<td>11.3 ± 3.0</td>
<td>26.1 ± 3.3</td>
<td>0.066 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate oxidation (μmol·kgFFM⁻¹·min⁻¹)</td>
<td>4.5 ± 2.5</td>
<td>7.3 ± 1.6</td>
<td>14.1 ± 2.3</td>
<td>0.050 ± 0.004*</td>
</tr>
<tr>
<td></td>
<td>Fat oxidation (μmol·kgFFM⁻¹·min⁻¹)</td>
<td>10.1 ± 1.4</td>
<td>8.6 ± 1.2</td>
<td>14.1 ± 2.3</td>
<td>0.069 ± 0.007</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *P < 0.05, significantly different than no HRT.
The mechanisms by which estrogen treatment may alter insulin action in humans is not completely understood. We did not find differences between groups with respect to glucose oxidation rates, but insulin-stimulated glucose oxidation rates were different between the estrogen group and its comparison group. Glucose utilization was lower in the hormone-treated groups. In contrast to our results, animal studies would suggest that estradiol maintains or improves insulin sensitivity. Estrogen has been shown to increase glucose transport and glucose utilization in muscle cells of animals (41,42). Estrogen regulates insulin-induced glucose transport (43) through glucose transporter translocation in rat skeletal muscle (44). Moreover, in oophorectomized rats, there is a reduction in insulin-stimulated translocation of GLUT4 to the plasma membrane as well as a reduction in glycogen synthase protein expression in skeletal muscle, which contributes to a decrease in whole-body insulin sensitivity (44). We are unaware of any studies examining skeletal muscle glucose transport in postmenopausal women on or not on HRT. Based on the literature, which suggests that GLUT4 levels do not vary between normal lean glucose-tolerant and obese diabetic subjects (45), we would not expect differences in skeletal muscle GLUT4 protein between women taking estrogen, on HRT, or not on HRT. However, it is possible that glycogen synthase, GLUT4 translocation, and/or other early steps in the insulin-signaling pathway change are altered with the use of estrogen or estrogen plus progesterone in women. In addition, another potential mechanism of estrogen on insulin sensitivity could be mediated through estrogen's effect on peripheral vascular reactivity (46,47).

The menopause transition is a period of fat mass gain and loss of lean tissue (8). Moreover, an increase in central obesity occurs in women as they become postmenopausal (8). However, 2 years of HRT attenuates the increase in abdominal fat in postmenopausal women (12). Moreover, 6 months of HRT (estrogen followed by progesterone) reduces waist circumference and central abdominal fat by DXA, but not total body fat mass in women with type 2 diabetes (32). Even a shorter treatment of 3 months of transdermic estrogen plus medroxyprogesterone acetate resulted in a loss of body fat and a decrease in WHR (17). These studies suggest a selective reduction in central body fat with HRT. We hypothesized that the women taking estrogen or on HRT in our study would have lower central body fat and, thus, greater insulin sensitivity. On the contrary, the women taking estrogen or on HRT in our study did not have significantly lower levels of visceral or subcutaneous abdominal fat than women not on HRT. Small sample sizes may have reduced the power to detect differences be-
between groups. All women in our study were selected to be overweight and obese, and the matched groups had similar total body fat. None of the studies comparing insulin sensitivity between women on and not on HRT had previously measured visceral adiposity. Glucose disposal rate and endogenous glucose production are significantly correlated with total body fat, truncal fat, subcutaneous fat, and intra-peritoneal fat (21). Furthermore, the truncal subcutaneous fat and visceral fat are more significant determinants of insulin resistance than extremity subcutaneous fat (21,22). Our results concur with Brown et al. (19) that total body fat does not account for the differences in insulin sensitivity between women taking estrogen and those not on HRT and adds that differences in glucose utilization are evident despite similar central fat deposition. Because the women in all groups had comparable $V_{O_{2\text{max}}}$ values, it is unlikely that fitness level plays a role in the heightened insulin resistance observed in the groups treated with estrogen and estrogen plus progesterone.

Mid-thigh low-density lean tissue, a marker of fat content within and around the muscles (22), increases with age and inactivity and is associated with body fat and insulin resistance (22,25,48,49). Therefore, intramuscular fat could differ between postmenopausal women on and not on HRT and could contribute to variations in insulin resistance. In our study, there were no significant differences in mid-thigh low-density lean tissue between women taking estrogen and estrogen plus progesterone versus their respective matched women not on HRT. Therefore, we do not believe that intramuscular fat contributed to the greater insulin resistance in the overweight and obese women on estrogen and estrogen plus progesterone therapy. However, a larger sample of women on hormonal therapy is needed to confirm this finding.

In summary, our results show that overweight and obese women taking oral estrogen and those who combine estrogen plus progesterone are more insulin-resistant than nonhormone users, even when women are of comparable total body fat, abdominal adiposity, intramuscular fat, and physical fitness. Additional studies are needed to determine the cellular mechanisms that could account for the differences in insulin sensitivity between postmenopausal women who are taking estrogen, on combined hormonal therapy, or not on HRT.

Acknowledgments — This study was supported by National Institutes of Health Grants K01-AG00747 (A.S.R.), R29-A161066 (B.J.N.), K01-AG00685 (D.M.B.) and the Department of Veterans Affairs, Geriatrics Research, Education, and Clinical Center at Baltimore.

We thank the women who participated in this study, the nurses in the Geriatrics Services at the Baltimore VA Medical Center for technical assistance, and Adeola Donsumu and Dana Jones for laboratory assistance.

References