Effects of HRT and exercise training on insulin action, glucose tolerance, and body composition in older women

ELLEN M. EVANS,1 RACHAEL E. VAN PELT,2 ELLEN F. BINDER,1 DANIEL B. WILLIAMS,3 ALI A. EHSANI,1 AND WENDY M. KOHRT2
1Department of Internal Medicine, Division of Geriatrics and Gerontology, and 3Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri 63110; and 2Department of Medicine, Division of Geriatric Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262

Received 11 September 2000; accepted in final form 27 December 2000

Evans, Ellen M., Rachael E. Van Pelt, Ellen F. Binder, Daniel B. Williams, Ali A. Ehsani, and Wendy M. Kohrt. Effects of HRT and exercise training on insulin action, glucose tolerance, and body composition in older women. J Appl Physiol 90: 2033–2040, 2001.—The independent and combined effects of exercise training and hormone replacement therapy (HRT) on body composition, fat distribution, glucose tolerance, and insulin action were studied in postmenopausal women, aged 68 ± 5 yr, assigned to control (n = 19), exercise (n = 18), HRT (n = 15), and exercise + HRT (n = 16) groups. The exercise consisted of 2 mo of flexibility exercises followed by 9 mo of endurance exercise. HRT was conjugated estrogens 0.625 mg/day and trimonthly medroxyprogesterone acetate 5 mg/day for 13 days. Total and regional body composition were measured by dual-energy X-ray absorptiometry. Serum glucose and insulin responses were measured during a 2-h oral glucose tolerance test. There were significant main effects of exercise on reductions in total and regional (trunk, arms, legs) fat mass, increase in leg fat-free mass, and improvements in glucose tolerance and insulin action. There were significant main effects of HRT on the reduction of total fat mass (HRT, −3.0 ± 4.0 kg; no HRT, −1.3 ± 2.6 kg), with a strong trend for reductions in trunk and leg fat mass (both P = 0.07). There was also a significant improvement in insulin action in response to HRT. These results suggest that there are independent and additive effects of exercise training and HRT on the reduction in fat mass and improvement in insulin action in postmenopausal women; the effect of HRT on insulin action may be mediated, in part, through changes in central adiposity.

Hormone replacement therapy (HRT) attenuates the menopause-related increase in body fatness and abdominal adiposity (11, 13, 17, 33, 38). HRT has also been found to have beneficial effects on insulin action and glucose tolerance (6, 7, 36), although this is not a uniform finding (2, 5, 10, 15). It is possible that beneficial effects of HRT on insulin resistance are mediated through its actions on abdominal fat depots. Therefore, one aim of this study was to determine whether beneficial effects of HRT on insulin action are associated with reductions in central adiposity.

It is well established that endurance exercise training can improve glucose tolerance and reduce insulin resistance and central adiposity in older adults (16, 21, 35). However, the combined effects of exercise and HRT on glucose tolerance and insulin action in older women are unknown. An additional aim of the present study was to investigate the independent and combined effects of endurance exercise training and HRT on body composition, fat distribution, glucose tolerance, and insulin action in healthy, postmenopausal women.

METHODS

Subjects. The 68 women who completed the study were nonsmokers, aged 60–84 yr, had not used estrogen for at least 2 yr, and were sedentary. Participants were assigned to the following treatment groups: control (n = 19), exercise (n = 18), HRT (n = 15), and exercise + HRT (n = 16). Random assignment to treatment arms was not performed because the supervised exercise training sessions had to be coordinated with other exercise intervention studies being conducted within the division. To minimize the limitations imposed by nonrandom assignment, all participants met inclusion criteria for the study and were willing and able to participate in an exercise program and take HRT. Thirty-six of the participants (8 control, 10 exercise, 6 HRT, 12 exercise + HRT) also participated in a study of the effects of HRT on bone mineral density and body composition (17). All of the participants provided written, informed consent to participate in the study, which was approved by the Washington University Institutional Review Board.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Screening tests included a medical history, physical examination, chest X-ray, blood and urine chemistries, graded exercise test with monitoring of blood pressure and electrocardiogram, gynecological examinations, and a mammogram. Volunteers were excluded from participation if they had medical problems that contraindicated HRT or exercise.

**Diet evaluation.** Participants completed 7-day food records at the beginning and end of the intervention. A registered dietician instructed the participants on the procedures for weighing and recording foods in household measures and conducted interviews after food records were completed to validate their accuracy. Subjects were instructed to maintain their usual eating habits during the period of study. Records were analyzed by using Nutritionist IV (N-Squared Computing, Salem, OR).

**HRT.** HRT consisted of continuous conjugated estrogens (0.625 mg/day) and medroxyprogesterone acetate (MPA; 5 mg/day), for 13 consecutive days every third month (Wyeth-Ayerst, Philadelphia, PA). Because we were primarily interested in the effects of estrogens, the intent of the hormone replacement regimen was to use the minimal effective progestin dose that provides protection against the development of endometrial hyperplasia. In this respect, trimonthly cycling of MPA has been shown to reduce the incidence of endometrial hyperplasia to <2% (12, 45). In the exercise + HRT group, HRT was initiated at the start of the exercise program.

**Exercise program.** The exercise intervention consisted of a 2-mo, low-intensity conditioning program that focused on range of motion and flexibility, followed by 9 mo of weight-bearing exercise training. Participants were required to attend a minimum of three exercise sessions per week. The initial flexibility training was designed to reduce the likelihood of injury in the subsequent, more vigorous endurance exercise program.

The 9-mo endurance exercise program, as previously described (17), consisted of walking (treadmills, indoor track), jogging (treadmills, indoor track), and stair climbing and descending. Exercise prescriptions were individualized and updated on a weekly basis. The initial goal was to walk 30 min at a moderate intensity corresponding to ~70% of maximal heart rate. Subsequently, the exercise prescription was increased via intensity and duration, depending on individual tolerance, with the ultimate goal being 45 min of exercise at 80–85% of maximal heart rate during the last 3 mo of the exercise program.

**Maximal aerobic power.** Maximal aerobic power (V\(\text{O}_2\) max) was assessed as previously described (19) before and after the treatment period in all subjects. Additional assessments were made in exercising subjects at 3-mo intervals during the treatment period.

**Body composition and anthropometric measures.** Total and regional body composition (fat mass and fat-free mass) were determined by dual energy X-ray absorptiometry (DXA) using a Hologic QDR-1000/W instrument (version 5.64, enhanced whole body software, Hologic, Waltham, MA). The standard recommendations of the manufacturer were used to define the arm, leg, and trunk regions. Waist circumference was measured as the minimum circumference between the top of the iliac crest and the distal end of the rib cage along the midaxillary line. Waist area was calculated from waist circumference, with the assumption that the geometric shape of the cross-sectional waist area is a circle.

**Oral glucose tolerance test.** The 75-g oral glucose tolerance test (OGTT) was administered in the morning after a 12- to 14-h fast. To minimize potential deleterious effects of progestins on glucose tolerance, the postintervention OGTT was administered at least 3 wk after a MPA cycle. In exercising subjects, the postintervention OGTT was performed 14–19 h after an exercise session. Diet was monitored for 3 days before an OGTT to ensure an intake of at least 150 g/day of carbohydrates. Blood samples for the determination of glucose and insulin concentrations were obtained before and 30, 60, 90, and 120 min after ingestion of the glucose beverage. The total areas under the glucose and insulin curves (GluAUC and InsAUC, respectively) were calculated using the trapezoidal rule. The GluAUC \times InsAUC product was calculated as an index of insulin action (24, 26). Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA), and serum insulin concentrations were measured by a double-antibody radioimmunoassay (28).

**Statistical analysis.** Differences among groups in baseline measurements were evaluated using one-way ANOVA. The changes that occurred in response to exercise and/or HRT were evaluated by two-way (exercise \(\times\) HRT) ANOVA. Sequential stepwise multiple-regression analyses were used to determine the relationships among variables of interest. Statistical significance was defined as an alpha level \(\leq 0.05\). Data are reported as means \pm SD except in the figures, which reflect means \pm SE.

**RESULTS**

There were no significant differences among the groups at baseline in parameters of interest (Table 1). Total energy intake averaged 1,679 \pm 290, 1,738 \pm 206, 1,724 \pm 275, and 1,782 \pm 343 kcal/day in the control, exercise, HRT, and exercise + HRT groups, respectively, at the beginning of the study (\(P = \text{ not significant}\)). Macronutrient composition was similar among the groups, averaging 51–53% carbohydrate, 30–32% fat, and 16% protein. At the end of the study, the reported energy intake was increased by 79 \pm 271, 30 \pm 227, 150 \pm 299, and 52 \pm 195 kcal/day in the control, exercise, HRT, and exercise + HRT groups.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
<th>Control (n = 19)</th>
<th>Exercise (n = 18)</th>
<th>HRT (n = 15)</th>
<th>Exercise + HRT (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>69.6 \pm 6</td>
<td>66.3 \pm 3</td>
<td>70.7 \pm 7</td>
<td>66.4 \pm 4</td>
</tr>
<tr>
<td>Menopause age, yr</td>
<td>49.5 \pm 4</td>
<td>49.6 \pm 6</td>
<td>49.5 \pm 5</td>
<td>49.5 \pm 5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.9 \pm 0.1</td>
<td>160.0 \pm 0.0</td>
<td>168.0 \pm 0.0</td>
<td>159.8 \pm 0.8</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>66.3 \pm 1.0</td>
<td>63.3 \pm 1.2</td>
<td>70.0 \pm 1.3</td>
<td>69.9 \pm 12.9</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>27.7 \pm 2.0</td>
<td>24.6 \pm 8.4</td>
<td>30.6 \pm 10.4</td>
<td>31.6 \pm 11.0</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>38.6 \pm 4.0</td>
<td>38.1 \pm 4.0</td>
<td>39.1 \pm 5.0</td>
<td>38.3 \pm 4.3</td>
</tr>
<tr>
<td>Waist girth, cm</td>
<td>80.1 \pm 7.1</td>
<td>79.6 \pm 9.8</td>
<td>86.6 \pm 13.6</td>
<td>84.7 \pm 13.2</td>
</tr>
<tr>
<td>Waist fat, kg</td>
<td>12.8 \pm 4.5</td>
<td>11.3 \pm 5.3</td>
<td>14.6 \pm 6.6</td>
<td>14.7 \pm 6.4</td>
</tr>
<tr>
<td>(\text{V}O_2\text{max}), ml·kg(^{-1})·min(^{-1})</td>
<td>19.9 \pm 3.0</td>
<td>21.7 \pm 3.7</td>
<td>18.3 \pm 4.0</td>
<td>20.2 \pm 3.2</td>
</tr>
<tr>
<td>Fasting Glu, mg/dl</td>
<td>95.0 \pm 11.1</td>
<td>96.6 \pm 14.2</td>
<td>101.1 \pm 29.1</td>
<td>96.3 \pm 11.5</td>
</tr>
<tr>
<td>Fasting Ins, (\mu)U/ml</td>
<td>7.2 \pm 3.2</td>
<td>5.7 \pm 2.1</td>
<td>7.3 \pm 3.9</td>
<td>8.2 \pm 4.5</td>
</tr>
<tr>
<td>Glu area, mg/dl·min(^{-1})</td>
<td>18.0 \pm 4.3</td>
<td>17.2 \pm 3.6</td>
<td>19.1 \pm 6.5</td>
<td>17.4 \pm 4.6</td>
</tr>
<tr>
<td>Ins area, (\mu)U/ml·min(^{-1})</td>
<td>7.3 \pm 3.7</td>
<td>5.7 \pm 2.2</td>
<td>5.9 \pm 2.1</td>
<td>8.8 \pm 5.2</td>
</tr>
<tr>
<td>Glu \times Ins (\times10(^{5}))</td>
<td>13.0 \pm 8.4</td>
<td>11.0 \pm 4.9</td>
<td>11.0 \pm 5.2</td>
<td>16.0 \pm 11.0</td>
</tr>
</tbody>
</table>

Values are means \pm SD. HRT, hormone replacement therapy; \(\text{V}O_2\text{max}\), maximal aerobic power; Glu, glucose; Ins, insulin.
respectively ($P = n$ significant for changes within and between groups); macronutrient composition was unchanged.

**Exercise training.** The exercise groups performed a similar amount of exercise. Subjects in the exercise group attended $3.5 \pm 0.7$ sessions/wk and exercised $45 \pm 6$ min/day at a heart rate of $126 \pm 15$ beats/min, corresponding to $78 \pm 7\%$ of maximal heart rate. Similarly, subjects in the exercise + HRT group attended $3.5 \pm 0.7$ sessions/wk and exercised $46 \pm 6$ min/day at a heart rate of $132 \pm 10$ beats/min, corresponding to $80 \pm 5\%$ of maximal heart rate. The exercise and exercise + HRT groups had similar improvements in $\text{VO}_{2\text{max}}$ of $19.5 \pm 15.0$ and $15.6 \pm 17.1\%$, respectively (both $P < 0.01$). There were no significant changes in $\text{VO}_{2\text{max}}$ in the control ($-2.2 \pm 7.3\%$) or HRT ($-3.7 \pm 11.1\%$) groups.

**Total and regional fat-free mass** (Table 2, Figs. 1 and 2). The only measure for which there was a significant exercise $\times$ HRT interaction was the change in arm fat mass, which decreased significantly only in response to exercise + HRT (different from all other groups, $P < 0.05$). Changes in arm fat mass in the control, exercise, HRT, and exercise + HRT groups were $0.1 \pm 0.4$, $-0.1 \pm 0.4$, $0.1 \pm 0.6$, and $-0.6 \pm 0.5$ kg, respectively. There were significant main effects of exercise for the reductions in hip waist area and total, trunk, and leg fat mass (all $P < 0.001$). There was a significant main effect of HRT for the reduction in total fat mass ($P < 0.05$), with strong trends for reductions in trunk and leg fat mass (both $P = 0.07$).

At baseline, trunk fat comprised $45 \pm 7$, $44 \pm 7$, $46 \pm 6$, and $46 \pm 6\%$ of total fat mass in the control, exercise, HRT, and exercise + HRT groups, respectively, and the largest absolute reductions in response to exercise or exercise + HRT were from this region (Table 2). Moreover, the relative reduction in trunk fat mass was also significantly larger ($-9 \pm 14\%$) than the relative reductions in arm ($-2 \pm 16\%$; $P < 0.001$) or leg fat mass ($-4 \pm 9\%$; $P < 0.001$), indicating a disproportionate loss of central body fat in response to exercise and/or HRT.

**Total and regional fat-free mass** (Table 2, Figs. 1 and 2). There were no exercise $\times$ HRT interactive effects on any of the measures of fat-free mass, nor were there any significant main effects of HRT. There were significant main effects of exercise for increases in trunk ($P < 0.05$) and leg fat-free mass ($P < 0.001$). There was a strong trend for trunk fat-free mass to be increased, in

Table 2. **Main effects of exercise and HRT on changes in total and regional body composition and Glu and Ins responses to OGTT**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Exercise (n = 34)</th>
<th>No exercise (n = 34)</th>
<th>P value</th>
<th>HRT (n = 31)</th>
<th>No HRT (n = 37)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FM, kg</td>
<td>$-3.8 \pm 3.5$</td>
<td>$-0.3 \pm 2.1$</td>
<td>&lt;0.001</td>
<td>$-3.0 \pm 4.0$</td>
<td>$-1.3 \pm 2.6$</td>
<td>0.03</td>
</tr>
<tr>
<td>Trunk FM, kg</td>
<td>$-2.4 \pm 2.3$</td>
<td>$-0.3 \pm 1.3$</td>
<td>&lt;0.001</td>
<td>$-1.9 \pm 2.4$</td>
<td>$-1.0 \pm 1.8$</td>
<td>0.07</td>
</tr>
<tr>
<td>Leg FM, kg</td>
<td>$-1.1 \pm 1.2$</td>
<td>$0.0 \pm 0.9$</td>
<td>&lt;0.001</td>
<td>$-0.8 \pm 1.4$</td>
<td>$-0.3 \pm 0.9$</td>
<td>0.07</td>
</tr>
<tr>
<td>Arm FM, kg</td>
<td>$-0.3 \pm 0.5$</td>
<td>$0.1 \pm 0.5$</td>
<td>&lt;0.01</td>
<td>$-0.3 \pm 0.6$</td>
<td>$0.0 \pm 0.4$</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist area, cm$^2$</td>
<td>$-39 \pm 41$</td>
<td>$-1 \pm 44$</td>
<td>&lt;0.001</td>
<td>$-31 \pm 51$</td>
<td>$-13 \pm 40$</td>
<td>0.14</td>
</tr>
<tr>
<td>Total FFM, kg</td>
<td>$1.3 \pm 1.5$</td>
<td>$0.3 \pm 1.7$</td>
<td>0.02</td>
<td>$1.1 \pm 1.9$</td>
<td>$0.5 \pm 1.4$</td>
<td>0.14</td>
</tr>
<tr>
<td>Trunk FFM, kg</td>
<td>$0.7 \pm 1.1$</td>
<td>$0.2 \pm 1.1$</td>
<td>0.08</td>
<td>$0.7 \pm 1.3$</td>
<td>$0.2 \pm 0.9$</td>
<td>0.09</td>
</tr>
<tr>
<td>Leg FFM, kg</td>
<td>$0.6 \pm 0.7$</td>
<td>$0.0 \pm 0.5$</td>
<td>&lt;0.001</td>
<td>$0.3 \pm 0.7$</td>
<td>$0.2 \pm 0.7$</td>
<td>0.56</td>
</tr>
<tr>
<td>Arm FFM, kg</td>
<td>$0.1 \pm 0.3$</td>
<td>$0.1 \pm 0.4$</td>
<td>0.39</td>
<td>$0.1 \pm 0.4$</td>
<td>$0.1 \pm 0.4$</td>
<td>0.63</td>
</tr>
<tr>
<td>Fasting Glu, mg/dl</td>
<td>$-8 \pm 11$</td>
<td>$0 \pm 12$</td>
<td>&lt;0.01</td>
<td>$-7 \pm 10$</td>
<td>$-2 \pm 14$</td>
<td>0.10</td>
</tr>
<tr>
<td>Fasting Ins, $\mu$U/ml</td>
<td>$-1.8 \pm 3.2$</td>
<td>$-0.2 \pm 3.5$</td>
<td>&lt;0.05</td>
<td>$-1.9 \pm 4.0$</td>
<td>$-0.2 \pm 2.6$</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GluAUC</td>
<td>$-2.3 \pm 2.8$</td>
<td>$0.0 \pm 3.3$</td>
<td>&lt;0.01</td>
<td>$-1.4 \pm 2.9$</td>
<td>$-0.9 \pm 3.6$</td>
<td>0.61</td>
</tr>
<tr>
<td>InsAUC</td>
<td>$-2.7 \pm 2.9$</td>
<td>$0.0 \pm 2.5$</td>
<td>&lt;0.001</td>
<td>$-2.5 \pm 3.3$</td>
<td>$-0.4 \pm 2.4$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GluAUC $\times$ InsAUC</td>
<td>$-6.0 \pm 6.5$</td>
<td>$0.1 \pm 6.3$</td>
<td>&lt;0.001</td>
<td>$-5.3 \pm 7.2$</td>
<td>$-0.9 \pm 6.3$</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD. OGTT, oral glucose tolerance test; FM, fat mass; FFM, fat-free mass; GluAUC, integrated response to OGTT (mg/dl $\times$ min $^{-1}$); InsAUC, integrated insulin response to OGTT ($\mu$U/ml $\times$ min $^{-1}$); GluAUC $\times$ InsAUC, product of glucose and insulin response ($\times$10$^5$). *Significant exercise-by-HRT interaction effect, $P = 0.04$. 

![Fig. 1. Changes in fat mass, fat-free mass, and waist area in control subjects and in response to exercise, hormone replacement therapy (HRT), and exercise + HRT. *Significant main effect of exercise ($P < 0.05$); †significant main effect of HRT ($P < 0.05$).]
response both to exercise ($P = 0.08$) and HRT ($P = 0.09$).

**OGTT glucose and insulin responses** (Table 2 and Fig. 3). There was a significant main effect of exercise, but not HRT, to reduce Glu$_{AUC}$. In contrast, there were significant main effects of exercise ($P < 0.001$) and HRT ($P < 0.01$) on reducing both the Ins$_{AUC}$ and the Glu$_{AUC}$ × Ins$_{AUC}$ product.

**Associations of changes in body composition, fat distribution, and $V_O^{2\text{max}}$ with changes in glucose tolerance and insulin action.** Sequential stepwise multiple-regression analyses were used to determine the independent effects of changes in total fat mass, trunk fat mass, leg fat mass, fat-free mass, and $V_O^{2\text{max}}$ on changes in Glu$_{AUC}$, Ins$_{AUC}$, and the Glu$_{AUC}$ × Ins$_{AUC}$ product (Table 3). Waist area was selected to adjust for the nonsignificant differences in baseline waist girth among the groups (i.e., for a given change in waist circumference, the change in waist area is dependent on the initial circumference). Additionally, because visceral fat is typically measured as an area, it was theorized that waist area is a more relevant measure than waist circumference, particularly in terms of expressing change. Trunk fat mass was included in the model because DXA may be a more sensitive measure of changes in central adiposity than simple anthropometric measurements (38).

The change in waist area was the strongest determinant of change in Glu$_{AUC}$, accounting for 18% of the variance; the remaining independent contributions of changes in fat mass, trunk fat mass, leg fat mass, fat-free mass, and $V_O^{2\text{max}}$ were not significant. The change in waist area accounted for 29% of the variance in the change in Ins$_{AUC}$ and increased to 35% when the change in trunk fat mass was included in the model. Similarly, the change in waist area accounted for 31% of the variance in the change in Glu$_{AUC}$ × Ins$_{AUC}$ product and increased to 38% when the change in trunk fat mass was added to the model.

**Effects of exercise and HRT, adjusted for change in waist area, on glucose tolerance and insulin action.** To determine whether the effects of exercise and HRT on the glucose and insulin responses to the OGTT were mediated primarily through changes in waist area, we repeated the two-way ANOVA and included change in waist area as a covariate. Preliminary analyses were conducted to verify that the interactions between the covariate and independent variables were not significant, which is an underlying assumption for analysis of covariance. Only the interaction of the change in waist area and the effect of HRT on Glu$_{AUC}$ was significant.

After we adjusted for the change in waist area, the main effect of exercise on the change in Glu$_{AUC}$ was no longer significant ($P = 0.08$). However, there remained significant main effects of exercise and HRT on both the changes in Ins$_{AUC}$ (exercise, $P < 0.01$; HRT, $P < 0.01$) and the changes in Glu$_{AUC}$ × Ins$_{AUC}$ (exercise, $P < 0.01$; HRT, $P < 0.01$). These relationships are depicted graphically for the Ins$_{AUC}$ in Fig. 4. Figure 4 shows that, for a given change in waist area, the change in Ins$_{AUC}$ in HRT users is different from that in nonusers (left) and different in exercisers vs. nonexercisers (right).

![Fig. 2. Changes in regional (trunk, legs, arms) fat mass (top) and fat-free mass (bottom) in control subjects and in response to exercise, HRT, and exercise + HRT. *Significant main effect of exercise ($P < 0.001$); ‡significant interaction of exercise and HRT (exercise + HRT different from all other groups) ($P < 0.05$).](http://jap.physiology.org/)

![Fig. 3. Changes in the integrated glucose and insulin responses to an oral glucose tolerance test and the product of these areas, in control subjects and in response to exercise, HRT, and exercise + HRT. *Significant main effect of exercise ($P < 0.01$); †significant main effect of HRT ($P < 0.01$).](http://jap.physiology.org/)
DISCUSSION

The major new findings of this study included 1) a significant reduction in total fat mass in response to HRT; 2) significant improvements in InsAUC and the GluAUC × InsAUC product in response to HRT; and 3) independent and additive effects of exercise and HRT on the reduction of fat mass and improvements in InsAUC and the GluAUC × InsAUC product.

Effects of exercise and HRT on fat mass. The exercise training program resulted in significant reductions in total and regional fat mass. The reduction in total fat mass of -3.8 kg was larger than that reported in several other studies of older women (21, 22, 29). This presumably reflected the relatively long duration and high intensity of the exercise program, as there were no apparent reductions in energy intake. However, this must be interpreted cautiously due to the inaccuracy of the method used to assess energy intake and the fact that energy intake was assessed only at the beginning and end of the study.

There is some evidence, both from cross-sectional comparisons of exercisers and nonexercisers (20, 44) and from prospective exercise training studies (21, 31), that exercise preferentially influences fat depots in central, as opposed to peripheral, regions of the body. This is particularly important given that central adiposity is associated more strongly with metabolic dysfunction than is total adiposity (8). The results of the present study indicate that there was a preferential loss of central body fat in response to exercise training. Trunk fat mass comprised 44 and 46% of total fat mass for women in the exercise and exercise + HRT groups, respectively, before starting the exercise program, but 62 and 68% of the fat loss that occurred in those groups was from central depots.

There is growing evidence that estrogens modulate abdominal fat deposition in women. This evidence includes observations that menopause triggers an increase in central adiposity (30, 41) and that abdominal fat accumulation is attenuated in postmenopausal women by replacement of estrogens, alone or in combination with progestins (11, 13, 17, 33). We anticipated that, if there were an effect of HRT on fat mass in the present study, it would be most apparent in measures of central adiposity (i.e., trunk fat mass and waist area). In fact, we found that there was a signif-

Table 3. Multiple regression analyses of the effects of changes in FM, trunk FM, waist area, leg FM, and V̇O₂max on changes in glucose tolerance and insulin action

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Step</th>
<th>Variable Entered</th>
<th>Multiple R²</th>
<th>Partial Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FM</td>
<td>Trunk FM</td>
</tr>
<tr>
<td>GluAUC</td>
<td>0</td>
<td></td>
<td>0.32</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Waist</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Trunk</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>GluAUC × InsAUC</td>
<td>1</td>
<td>Waist</td>
<td>0.27</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Trunk</td>
<td>0.00</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Multiple correlation coefficient ($R^2$) was adjusted for sample size and the number of predictor variables. For partial correlations, the association with the dependent variable independent of the variance accounted for by predictor variables already entered into the regression model. GluAUC, area under the glucose curve; InsAUC, area under the insulin curve; GluAUC × InsAUC, product of glucose and insulin areas.
icant reduction in total fat mass in response to HRT but only trends for reductions in measures of central adiposity. This may simply reflect the fact that there is less variability in the measurement of total fat mass by DXA than in the measurement of regional adiposity by DXA or anthropology. Further studies employing soft tissue imaging to evaluate the effects of HRT on visceral fat mass are warranted. Lack of knowledge regarding the mechanisms by which estrogens influence regional fat deposition and mobilization (3, 39) makes it difficult to interpret the findings of the present study. Nevertheless, the results indicate that there were independent and additive effects of exercise and HRT on reducing fat mass.

**Effects of exercise and HRT on fat-free mass.** The increases in fat-free mass that occurred in the exercise groups may seem incongruent with the concept that endurance exercise training does not typically induce muscle hypertrophy. In that regard, it was not possible to determine whether the increase in fat-free mass specifically reflected an increase in muscle mass. However, stair climbing, which involves lifting the body weight, was an integral part of the exercise program and may have induced an increase in muscle mass. The finding that leg, but not arm, fat-free mass was increased in response to an exercise program that involved primarily lower extremity musculature supports this contention.

Estrogen receptors are present in skeletal muscle (32, 34) and may mediate the anabolic effects of estrogen supplementation on muscle that occur in some species (42). The question of whether estrogen has anabolic effects on skeletal muscle in humans remains controversial. There is evidence from cross-sectional (1) and prospective (40) studies that menopause is associated with an accelerated loss of lean tissue other than bone mineral. In our previous studies, long-term HRT (18 mo) resulted in an increase in fat-free mass in older women (17), but HRT did not significantly augment the increases in fat-free mass or muscle strength that occurred in response to exercise training (4, 17).

In the present study, there were no significant effects of HRT on total or regional fat-free mass. As in our laboratory’s previous studies (4, 17), the increase in fat-free mass that occurred in response to exercise was not augmented by HRT. Because estrogens promote fluid retention by upregulating osmotic secretion of arginine vasopressin and increasing renal sodium resorption (37), it seems likely that small increases in fat-free mass in response to HRT reflect an expansion of the water fraction of the fat-free mass.

**Effects of exercise and HRT on glucose tolerance and insulin action.** Abdominal obesity is a strong determinant of insulin resistance and glucose intolerance (8, 18). Both glucose tolerance and insulin action can be favorably affected by exercise, but the effect is short lived, lasting only about 48 h after an exercise bout (14). More persistent improvements in glucose tolerance and insulin action in response to exercise training occur as a result of reductions in fat mass, particularly in central depots (9, 23). The results of the present study confirm the beneficial effects of exercise on the glucose and insulin responses to OGTT and on the product of the glucose and insulin areas, which is an index of insulin action (26). The findings further corroborate the importance of reducing abdominal obesity, as the reduction in waist area explained 18% of the improvement in glucose tolerance and reductions in waist area and trunk fat mass explained 35 and 38% of the respective improvements in insulin area and insulin action.

The literature on the independent effects of estrogen on glucose tolerance and insulin resistance is equivocal and difficult to interpret due to numerous differences in treatment duration, small sample sizes, variation in methods of measuring insulin resistance, and different hormone replacement regimens (e.g., dose and type of estrogen; dose, type, and cycling of progestins; oral vs. transdermal). However, our results are consistent with those of several studies that found that oral glucose tolerance was unchanged in response to oral estrogens but that the insulin response to glucose was significantly reduced, indicating enhanced insulin action (6, 25, 27, 36). For example, the multicenter Menopause Study (25) of 525 women found that glucose tolerance was unchanged in response to unopposed estrogen but deteriorated in the groups treated with continuous (2.5 or 5.0 mg/day) or cyclic (5 or 10 mg/day for the last 14 days of 28-day cycles) MPA. Insulin areas, on the other hand, were significantly reduced, by up to 40%, in all of the treatment groups except the low-dose, continuous MPA group. Moreover, the decreases in insulin area became progressively larger with increasing duration of treatment (i.e., from 3 to 6 to 13 cycles). If estrogen does indeed play a role in the regulation of visceral fat metabolism, it would be plausible to hypothesize that the progressive reduction in hyperinsulinemia occurred as a result of a slow, steady decline in visceral adiposity.

Regardless of the mechanism of action, if estrogen replacement reduces hyperinsulinemia, it would be logical to assume that this would be accompanied by decreased insulin resistance. Yet among several recent studies (2, 5, 7, 10, 15) that used the hyperinsulinemic-euglycemic clamp procedure, which is the most rigorous method of assessing insulin resistance, most found that insulin action was not significantly increased in response to HRT (2, 5, 10, 15). However, the latter studies involved only 6–12 wk of HRT. If a reduction in visceral adiposity is the driving mechanism for improvement in insulin action in response to HRT, the periods of treatment were probably not of sufficient duration to achieve significant changes. Interestingly, in the study that found enhanced insulin action in response to estrogen replacement, the period of therapy was 24 wk and improvements occurred only in women who were initially hyperinsulinemic (7). In that study, insulin-stimulated glucose disposal increased by ~50%, and there was a 30% reduction in the insulin response to an oral glucose challenge, despite no change in the integrated glucose response. The improvement in insulin action did not appear to be re-
lated to a reduction in the waist-to-hip circumference ratio, but the latter measure is not a sensitive index of change in abdominal adiposity (43). In the present study, there remained significant main effects of HRT on the reductions in the insulin area and the glucose × insulin area product even after adjusting for reductions in waist area. Clearly, further studies are necessary to confirm the effects of long-term HRT on insulin action and elucidate the mechanisms of action.

As expected, exercise training resulted in significant reductions in total and regional fat mass, increases in total and leg fat-free mass, and improvements in glucose tolerance and insulin action. The major new findings of this study were that HRT and exercise training had independent and additive effects on the reduction of total fat mass, the attenuation of the insulin response to an OGTT, and the improvement in insulin action in older, postmenopausal women. The effects of HRT on insulin action were mediated, in part, by changes in central adiposity, as reductions in waist area and trunk fat mass explained almost 40% of the improvement in these outcomes. However, the effects of HRT on the OGTT insulin response and the glucose × insulin area product remained significant after adjusting for changes in waist area; the mechanism for this action of HRT is unknown.

We are grateful for the expert technical assistance and support provided by the staffs of the Division of Geriatrics and Gerontology, the General Clinical Research Center (RR-00036), and the Diabetes Research and Training Center (DR-20579). This research was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-40705 and by the General Clinical Research Center (RR-00036), and the Diabetes Research Center of the University of Washington Claude Pepper Older Americans Independence Center (AG-13629).

REFERENCES