Dietary restriction and walking reduce fat deposition in the midthigh in obese older women

Alice S Ryan, Barbara J Nicklas, Dora M Berman, and Karen E Dennis

ABSTRACT

Background: It is suggested that fat deposition within midthigh muscle, represented by low-density lean tissue, increases with deconditioning and obesity and is associated with risk factors for cardiovascular disease (CVD) in women.

Objective: We determined the effects of a 6-mo weight loss and walking (3 times/wk) program (WL+AEX) on midthigh low-density lean tissue and glucose and lipid metabolism in 24 sedentary, obese [body mass index (kg/m²): 32 ± 1 (x ± SEM)] postmenopausal women aged 58 ± 1 y.

Design: Total body fat and fat-free mass were measured by using dual-energy X-ray absorptiometry. Intraabdominal fat (IAF), subcutaneous abdominal fat (SAF), midthigh fat, midthigh muscle, and midthigh low-density lean tissue areas were measured by using computed tomography. Glucose and insulin responses were determined with a 3-h oral-glucose-tolerance test.

Results: Body weight decreased 8% (P < 0.001) and maximal aerobic capacity increased 8% (P < 0.001) with the weight loss and walking program. Total body fat decreased by 15% (P < 0.001) whereas fat-free mass did not change. IAF and SAF decreased by 18% and 16%, respectively (P < 0.001). Midthigh fat and midthigh low-density lean tissue decreased by 16% and 18%, respectively (P < 0.001), and midthigh muscle area increased by 7% (P < 0.05). Fasting plasma insulin decreased by 12% and total glucose and insulin areas under the curve decreased by 6% and 24%, respectively (P < 0.05). HDL-cholesterol concentrations increased 8% (P < 0.05) and triacylglycerol concentrations decreased 19% (P < 0.001).


KEY WORDS Obesity, computed tomography, fat-free mass, exercise, VO₂ max, maximal oxygen consumption, postmenopausal women

INTRODUCTION

A sedentary lifestyle and significant alterations in body composition promote the development of cardiovascular disease (CVD). Total and abdominal adiposity increase the risk of coronary artery disease and mortality among women (1, 2). Moreover, the age-associated increase in total and visceral adiposity may contribute to glucose intolerance (3) and the increase in triacylglycerol, total cholesterol, and LDL cholesterol with age (4, 5). Therefore, aging, inactivity, and total and central adiposity may predispose individuals to CVD, type 2 diabetes, and dyslipidemia (6, 7).

Muscle area, measured by computed tomography (CT), can be divided into areas of normal and low muscle attenuation. Muscle areas of low attenuation represent increased fat content in and around the muscle fibers (8). Lean tissue with a density below the normal range increases with age (9, 10) and obesity (11) in men. We showed previously that midthigh low-density lean tissue is directly related to age and adiposity in women (12). In addition, subjects with more midthigh low-density lean tissue have lower aerobic fitness (8, 12). Low muscle attenuation (ie, more fat deposition in the muscle) is also associated with insulin resistance and dyslipidemia in men and premenopausal women (8, 12, 13). One study recently reported decreases in midthigh low-density muscle with weight loss in young men and women (14). A reduction in physical fitness with age, as well as an increase in adiposity, is likely to contribute to the aging-associated increase in low-density lean tissue observed in muscle. We are unaware of any studies that examined the extent to which midthigh low-density lean tissue area is modifiable by changes in both physical conditioning and energy restriction. Furthermore, midthigh muscle area, which increases in older men after endurance exercise training (15), may also increase in older women after an endurance program.

The purpose of this study was to determine the effects of a 6-mo hypoenergetic weight loss and walking program on midthigh low-density lean tissue in obese postmenopausal women. We hypothesized that the reduction in the fat accretion in skeletal muscle area would increase midthigh muscle area and improve glucose and lipid metabolism in these older women.
Dietary restriction and exercise would be associated with an improvement in glucose and lipid metabolism.

SUBJECTS AND METHODS

Subjects

All subjects were healthy, overweight [body mass index (BMI; in kg/m²) > 27] women between the ages of 51 and 66 y who were ≥1 y past menopause and not receiving hormone replacement therapy. Only women who were weight stable (<2.0 kg weight change in the previous year) and sedentary (<20 min of aerobic exercise 2 times/wk for the previous 6 mo) were recruited. The subjects were screened by using a medical history questionnaire, a physical examination, a fasting blood profile, and a graded exercise treadmill test in an attempt to exclude those with CVD. All the subjects were nonsmokers; had no evidence of diabetes; hyperlipidemia; cancer; liver, renal or hematologic disease; or other medical disorders; and were not taking any medications known to influence body composition or glucose or lipid metabolism. Seventy-six women met all study criteria and were enrolled in the study. Seventeen women dropped out of the program because of illness, relocation, personal reasons, or time constraints. Twenty-four of the remaining 59 women had pre- and posttreatment data (CT scans) and are included in this report. All the women were white except 2 who were African American. All methods and procedures for the study were approved by the Institutional Review Board of the University of Maryland. Each participant provided written, informed consent.

Dietary restriction and walking program

The women first completed an initial 7-d food record to provide information about their dietary habits. To minimize the effect of changes in dietary composition during weight loss and walking on the measured metabolic variables, all subjects were instructed by a registered dietitian to follow the American Heart Association (AHA) Step I diet (16) for 6–8 wk before the initiation of the study. The composition of this diet was 50–55% carbohydrate, 15–20% protein, ≤30% fat, and ≤300 mg cholesterol/d. The women were weight stable (1–2 kg weight change) while following this diet for 2 wk before research testing. Compliance was monitored by weekly review of 7-d food records and 24-h dietary recalls. During the 6-mo weight loss intervention, the women attended weekly weight loss classes led by the registered dietitian for instruction in the principles of a hypoenergetic diet that follows the AHA guidelines. The women were instructed to restrict their energy intake by 1045–1465 kJ (250–350 kcal)/d. The program focused on eating behavior, stress management, control of portion sizes, and modification of binge eating and other adverse habits and encouraged low-intensity walking 3 d/wk. The subjects walked 1 d/wk on a treadmill at our exercise facility at 50–60% heart rate reserve for 30–45 min under the supervision of an exercise physiologist and were instructed to walk the other 2 d on their own. At the end of the 6-mo program, the women were weight stabilized (0.5 kg change) on a euenergetic diet for 2 wk before final testing and were asked to continue walking 3 d/wk during the final testing period.

Maximal oxygen uptake

Maximal oxygen uptake (VO₂max) was measured before and after the exercise training by using a continuous treadmill test protocol described previously (17). 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 VO₂max included meeting 2 of the following 3 criteria: 1) a plateau in oxygen uptake with an increased work load 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. 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 the waist-to-hip ratio (WHR).

Dual-energy X-ray absorptiometry

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

Computed tomography

To quantify visceral and abdominal subcutaneous fat areas, a CT scan of the abdomen was performed by using a PQ6000 scanner (Marconi Medical Systems, Cleveland, OH). A single, 5-mm scan was taken at the L4–L5 region while the subject was supine with her arms stretched above her head. A fat tissue–highlighting technique was used to quantitate the relative proportions of intraabdominal adipose tissue (IAF) and subcutaneous adipose tissue areas. Sagittal diameter was determined 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 midthigh was used to quantify muscle area (HU: 30 to 80), total fat area of the thigh (HU: −190 to −30), and low-density lean tissue (HU: 0 to 29) of both the right and the left legs as described previously (12).

Metabolic testing

All tests were performed in the morning after a 12-h overnight fast. All subjects were weight stabilized before metabolic testing and blood samples were drawn 36–48 h after the last bout of walking.

Oral-glucose-tolerance test

All subjects underwent a 75-g, 3-h oral-glucose-tolerance test (18). Blood samples were drawn every 30 min during a 3-h period for measurement of plasma glucose and insulin concentrations. Plasma glucose concentrations were determined by using the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Blood samples were collected into chilled tubes containing 1.5 g EDTA/L blood in a total volume that was 4% of the sample volume. The blood samples were centrifuged at 4°C and 1500 × g for 15 min and a
TABLE 1

<table>
<thead>
<tr>
<th>Subject characteristics before and after weight loss and walking*</th>
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<tr>
<td><strong>Before</strong></td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Body weight (kg)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist circumference (cm)</td>
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<td>Hip circumference (cm)</td>
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<tr>
<td>Thigh circumference (cm)</td>
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<tr>
<td>WHR</td>
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<tr>
<td>VO₂max (mL·kg·min⁻¹)</td>
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<td>VO₂max (L/min)</td>
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*2 ± SEM; n = 24. WHR, waist-to-hip ratio; VO₂max, maximal oxygen consumption.

Significantly different from before the intervention: ²P < 0.001, ¹P < 0.05.

1-mL aliquot of plasma was rapidly frozen (−80°C) for subsequent hormone analysis. All determinations were performed in duplicate. Immunoactive insulin was determined by radioimmunoassay with use of 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). The lower limit of detection of this assay in our laboratory is 12 pmol/L. Intra- and interassay CVs of pooled control sera average 5% and 9%, respectively.

**Lipoprotein lipids**

The average of 2–3 blood draws, which were taken on different days, was used in the determination of lipoprotein lipids. Blood samples were transferred into chilled tubes containing 1 g EDTA/L blood. Plasma was separated by centrifugation at 4°C for 15 min at 2000 × g. Total cholesterol, triacylglycerol, HDL cholesterol, HDL₂ cholesterol and HDL₃ cholesterol were measured as described previously (12, 19) and LDL cholesterol was calculated by using the Friedwald equation (20). In our laboratory, the inter- and intraassay CVs for the measurement of total cholesterol were 6.2% and 1.5%, for triacylglycerol were 7.6% and 2.6%, and for HDL cholesterol were 9.2% and 2.7%, respectively.

**Statistical analyses**

The values of the right leg were used in the statistical analyses for mid thigh muscle area, fat area, and low-density lean tissue area. Total 3-h glucose and insulin areas were calculated by using the trapezoidal method (21). Changes with weight loss and walking for the dependent variables were calculated by using paired t tests. Relations between variables were determined by linear regression analyses and calculation of Pearson correlation coefficients. Statistical significance was set at P < 0.05 for all tests. All data were analyzed by using SPSS statistical software (SPSS Inc, Chicago). All values are expressed as means ± SEMs.

**RESULTS**

**Subject characteristics**

The characteristics of the women before and after weight loss and walking are presented in Table 1. WHR was > 0.80 in all but 8 of the women, indicating that most of these women had an upper-body fat distribution. Body weight and BMI decreased by 8% (P < 0.001). Waist and hip circumferences decreased by 4% (P < 0.001 for both); thus, there was no change in the WHR. VO₂max (L/min) increased 8% after the walking intervention (P < 0.01).

**Body composition**

The changes in total and regional body composition with weight loss and exercise showed an absolute decrease in percentage body fat of 4% and a 15% decrease in total fat mass (P < 0.001), whereas there was no significant change in FFM (Table 2). IAF and subcutaneous abdominal adipose tissue decreased by 18% and 16%, respectively (P < 0.001). This was accompanied by an ∼2-cm reduction in sagittal diameter of the abdomen (P < 0.001).

There was a significant 4% decrease in the circumference of the mid thigh (P < 0.001) by anthropometry. There were also significant changes in mid thigh lean and fat tissue by CT analysis. Mid thigh low-density lean tissue decreased by 18%, mid thigh fat decreased by 16% (P < 0.001), and mid thigh muscle area increased by 7% after weight loss and walking (P < 0.05).

**Glucose and lipid metabolism**

Ten women (42%) had impaired glucose tolerance at baseline. The percentage of women with impaired glucose tolerance decreased to 17% (n = 6) after the weight loss and walking program (Table 3). Weight loss and walking did not change fasting glucose concentrations but the 2-h glucose concentration decreased by 10% (7.1 ± 0.4 compared with 6.2 ± 0.3 mmol/L) and the 3-h glucose area decreased by 8% (P < 0.05). There were also significant reductions in plasma insulin concentrations after weight loss and walking: fasting insulin concentrations decreased by 12% (P < 0.05) and the 3-h area under the insulin curve decreased by 24% (P < 0.01). Weight loss and walking reduced plasma triacylglycerol concentrations by 19% (P < 0.001) but did not significantly change total cholesterol and LDL-cholesterol concentrations. Plasma HDL-cholesterol concentrations increased by 7% (P < 0.05) because of increases in HDL₂ cholesterol (P < 0.01), with no significant change in HDL₃ cholesterol.

**Relations with mid thigh body composition at baseline**

At baseline, mid thigh muscle area correlated positively with VO₂max (r = 0.58, P < 0.01). Mid thigh fat correlated with total body fat mass (r = 0.57) and thigh circumference (r = 0.63), whereas mid thigh muscle area correlated with total-body FFM at baseline (r = 0.64, all P < 0.01). Mid thigh low-density lean tissue did not correlate with measures of body composition, VO₂max, or glucose and lipid metabolic risk factors.

The change in mid thigh low-density lean tissue with weight loss and walking correlated with baseline mid thigh low-density lean tissue (r = −0.76, P < 0.01; Figure 1). The change in mid thigh low-density lean tissue was inversely related to the change in mid thigh muscle area (r = −0.41, P < 0.05). Initial mid thigh low-density lean tissue correlated positively with changes in mid thigh muscle area (r = 0.42, P < 0.05). In addition, the change in 3-h area under the glucose curve correlated with baseline mid thigh low-density lean tissue (r = −0.48, P < 0.05). The changes in glucose and lipid metabolism variables did not correlate with changes in low-density lean tissue. Thus, women with the most mid thigh low-density lean tissue at baseline lost the most low-density lean tissue, had the greatest gains in mid thigh muscle, and had the largest improvements in glucose metabolism with weight loss and walking.
Weight loss selectively depleted low-density skeletal muscle because there was no change in the amount of skeletal muscle with normal attenuation values (14). This loss of low-density skeletal muscle with weight loss alone was similar to the loss in low-density lean tissue that we observed. Yet, the amount of weight loss in that study was twice as great as in this one. This suggests that the walking program may have contributed to the reduction in midthigh low-density lean tissue. However, with the present study design, we were unable to test whether the weight loss or the exercise intervention resulted in a greater decline in midthigh low-density lean tissue or whether the effects of these interventions were additive or synergistic.

The loss of midthigh low-density lean tissue correlated with initial midthigh low-density lean tissue, suggesting that women with the greatest amount of fat deposition in muscle have the largest reductions in this tissue. Moreover, those with the highest low-density lean tissue at baseline had the greatest reductions in glucose areas under the curve. This suggests that the physical deconditioning and metabolic abnormalities associated with low-density lean tissue can be improved with weight loss and exercise. In addition, the change in midthigh low-density lean tissue correlated with the change in midthigh muscle area, indicating that the women who lost the most midthigh low-density lean tissue also showed the greatest increases in thigh muscle area. Thus, the reduction of fat in and around muscle with subsequent gains in muscle itself are beneficial body-composition adaptations to a weight loss program that enhance the health of obese postmenopausal women.

The weight loss and walking program also resulted in reductions in abdominal and midthigh fat and increases in midthigh muscle. These findings substantiate those of Despres et al (23), who showed a greater loss of abdominal fat than midthigh fat after a 14-mo exercise training program in obese postmenopausal women. A loss of total and abdominal body fat has been reported consistently after aerobic exercise or exercise and weight loss (23–26). FFM usually does not change with aerobic exercise (23, 25) or exercise combined with weight loss (24, 26–28) but declines with weight loss alone (24, 29). In contrast, we observed a significant increase in midthigh muscle area in postmenopausal women as a result of walking that was similar to that reported in men (15). Because both aging and weight loss are associated with a loss of FFM (30), the small increase in

### TABLE 2

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<thead>
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<tr>
<td>Total body</td>
<td></td>
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<tr>
<td>Body fat (%)</td>
<td>48.3 ± 0.9</td>
<td>43.9 ± 1.1</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>40.6 ± 1.6</td>
<td>34.5 ± 1.6</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>43.1 ± 1.0</td>
<td>43.4 ± 0.9</td>
</tr>
<tr>
<td>Abdominal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraabdominal fat (cm²)</td>
<td>156.4 ± 8.8</td>
<td>128.7 ± 8.4</td>
</tr>
<tr>
<td>Subcutaneous fat (cm²)</td>
<td>493.3 ± 21.4</td>
<td>415.8 ± 19.8</td>
</tr>
<tr>
<td>Sagittal diameter (mm)</td>
<td>25.9 ± 0.5</td>
<td>23.9 ± 0.5</td>
</tr>
<tr>
<td>Midthigh</td>
<td></td>
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</tr>
<tr>
<td>Fat (cm²)</td>
<td>192.6 ± 8.7</td>
<td>162.5 ± 9.5</td>
</tr>
<tr>
<td>Muscle (cm²)</td>
<td>75.4 ± 2.8</td>
<td>79.6 ± 2.1</td>
</tr>
<tr>
<td>Low-density lean tissue (cm²)</td>
<td>20.8 ± 1.7</td>
<td>15.6 ± 1.1</td>
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1,2 SEM; n = 24.
3 Significantly different from before the intervention: *P < 0.001
4 *P < 0.05

### DISCUSSION

Muscle with lower than normal attenuation by CT scan is a body-composition characteristic that increases with age, reduced physical conditioning, and increased body fatness, and may contribute to the metabolic dysfunction associated with the insulin resistance syndrome (8, 9, 11, 12). The results of this study show that in overweight postmenopausal women, a combined weight loss and walking program reduces midthigh low-density lean tissue, increases midthigh muscle area, and improves CVD risk factor profiles.

Our previous study showed that midthigh low-density lean tissue in women is associated with increased total and central adiposity and reduced physical fitness, independent of age (12). This suggests that the muscle area of the thigh contains more adipose tissue in older, overweight, physically inactive women and that improvements in physical fitness and weight loss could decrease midthigh low-density lean tissue. The current study showed a significant loss of midthigh low-density lean tissue after a weight loss and walking intervention in overweight postmenopausal women. Moreover, the 18% reduction in midthigh low-density lean tissue was as great as the loss of visceral fat (18%), and slightly higher than both the decrease in subcutaneous abdominal fat and midthigh fat (both 16%). We also observed that the total area reduction in the midthigh by CT analysis was greater than that obtained by measuring changes in thigh circumference. These results suggest that anthropometric measurement was not as accurate as CT in determining changes in body composition that resulted from the dietary restriction and walking program.

The reduced attenuation of muscle is largely the result of increased fat content in and around muscle fibers (8). Exercise increases the capacity of muscle for lipid utilization (22), which may partially explain the loss of low-density lean tissue with the increase in aerobic capacity in the current study. In addition to the walking program, the diet-induced weight loss also contributed to the reduction in midthigh low-density lean tissue. To date, only one study has examined the changes in midthigh low-density skeletal muscle with diet-induced weight loss, and the results showed that an 15-kg weight loss resulted in a 28% reduction in midthigh fat and a 23% loss in midthigh low-density muscle in young men and women (14). Moreover, the

### TABLE 3

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<thead>
<tr>
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<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.09</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>74 ± 6</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>3-h Glucose area under the curve (mmol·min/L)</td>
<td>1235 ± 56</td>
<td>1138 ± 45</td>
</tr>
<tr>
<td>3-h Insulin area (pmol·min/L)</td>
<td>76.504 ± 9252</td>
<td>55.610 ± 5812</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.21 ± 0.19</td>
<td>5.08 ± 0.19</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.35 ± 0.17</td>
<td>3.28 ± 0.17</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.19 ± 0.05</td>
<td>1.27 ± 0.04</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>0.12 ± 0.02</td>
<td>0.19 ± 0.02</td>
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1,2 SEM; n = 24.
3 Significantly different from before the intervention: *P < 0.05
4 *P < 0.01.
midthigh muscle area found with walking may be important in the prevention of sarcopenia in older obese women.

Note that the women in our study had 2–3 times the amount of subcutaneous midthigh fat relative to midthigh muscle as measured by CT. This supports the results of our previous study (12) as well as those of an earlier study (31) in sedentary women who were older but who had less total body fat. In older men, however, the amount of fat in the midthigh is approximately one-third that of midthigh muscle (15, 32). This suggests that there are sex differences in relative amounts of muscle and fat in the midthigh in older persons.

Midthigh low-density lean tissue is related to hyperinsulinemia, hyperleptinemia, and dyslipidemia, but not independently of adiposity or age (12). In the present study, baseline low-density lean tissue did not correlate with baseline insulin, glucose, or lipoprotein lipid values, partially because the women were recruited to be very similar with respect to age and body fat. Insulin-stimulated glucose uptake is negatively correlated with low-density lean tissue (8, 13), and type 2 diabetes is associated with low muscle attenuation (11). Improvements in glucose and lipid metabolism did not correlate with the reduction in low-density lean tissue after weight loss and walking. This confirms the findings of Goodpaster et al (14), in which increases in glucose utilization after weight loss did not correlate with the loss of low-density skeletal muscle.

As discussed previously (12), measuring muscle fat with a single-slice CT scan has its limitations. First, we did not measure a volume of muscle or fat and cannot extrapolate the findings to the entire leg area. Second, we cannot discern intra- from extracellular fat by CT. A muscle biopsy would be required to assess the fat content in the muscle. Moreover, our results are applicable only to white, older postmenopausal women.

In summary, improvements in physical fitness and weight loss reduced midthigh low-density lean tissue, with coinciding improvements in glucose and lipid metabolic risk factors for type 2 diabetes and CVD in postmenopausal women.

We extend our appreciation to the women who participated in this study. We are grateful to the exercise physiologists who assisted in the training of the women, to Linda Bunyard and Naomi Tomoyasu for their dietary and behavior expertise and instruction, to the nurses in the Geriatrics Services at the Baltimore Veterans Affairs Medical Center for technical assistance, and to Andrew Goldberg for his support and insightful comments.

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