

Aerobic Exercise Is Necessary to Improve Glucose Utilization with Moderate Weight Loss in Women

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Abstract

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Objective: To determine the effects of weight loss (WL) alone and combined with aerobic exercise on visceral adipose tissue (VAT), intramuscular fat, insulin-stimulated glucose uptake, and the rate of decline in free fatty acid (FFA) concentrations during hyperinsulinemia.

Research Methods and Procedures: We studied 33 sedentary, obese (BMI = 32 ± 1 kg/m²) postmenopausal women who completed a 6-month (three times per week) program of either WL alone ($n = 16$) or WL + aerobic exercise (AEX) ($n = 17$). Glucose utilization (M) was measured during a 3-hour hyperinsulinemic-euglycemic clamp (40 mU/m² per minute). M/I, the amount of glucose metabolized per unit of plasma insulin (I), was used as an index of insulin sensitivity.

Results: Body weight, total fat mass, and percentage fat decreased similarly in both groups ($p < 0.01$). VAT, subcutaneous abdominal adipose tissue, mid-thigh subcutaneous fat, and intramuscular fat decreased to a similar extent in both groups and between 14% and 27% after WL and WL+AEX ($p < 0.05$). WL alone did not change M or M/I; however, M and M/I increased 15% and 21% after

WL+AEX ($p < 0.05$). Fasting concentrations and rate of decline of FFA did not change in either group. In stepwise regression models to determine the independent predictors of changes in M and M/I, the change in VAT was the single independent predictor of M ($r^2 = 0.30$) and M/I ($r^2 = 0.33$).

Discussion: Intramuscular fat decreases similarly with 6 months of moderate WL alone or with aerobic exercise in postmenopausal women. In contrast, only WL combined with exercise results in increased glucose utilization and insulin sensitivity. These findings should be validated in a larger population.

Key words: insulin resistance, visceral fat, intramuscular fat, weight loss, fat oxidation

Introduction

Approximately 50% to 65% of adults in the United States are classified as overweight, 20% to 25% are considered obese (1), and these numbers continue to escalate. Menopause is a period of increased fat deposition of the total body and abdominal region (2). Thus, early interventions designed to reduce obesity in women who have made the transition into menopause are important to reduce the risk of cardiovascular disease, hypertension, and type 2 diabetes. Both dietary restriction and increasing physical activity are promoted to combat obesity and its associated comorbidities. Current estimates indicate that 44% of women are attempting to lose weight, with less than one half of these women both reducing energy intake and exercising ≥ 150 min/wk (3). Moreover, only 21% of adults who walk for physical activity do so at the level recommended by public health guidelines, with the prevalence of recommended exercise being even lower among the obese (4).

Aging is associated with increased total and abdominal visceral fat and intramuscular fat in women (5); these increases are associated with both insulin resistance (6,7) and an impairment in insulin's ability to suppress lipolysis (8–

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10). Fasting free fatty acid (FFA)¹ levels are also implicated in the pathogenesis of insulin resistance (11). Furthermore, the rate of decline in FFAs during hyperinsulinemia is delayed in obesity and associated with a reduction in glucose uptake (12). Thus, declines in body fat and improvements in insulin's anti-lipolytic effect may potentially mediate an improvement in insulin sensitivity.

A decline in intramuscular fat is observed after weight loss (WL) alone (13,14) or when combined with moderate walking (15), but less is known concerning a direct comparison of WL alone or WL with high-intensity aerobic training. To date, only three studies (16–18) directly compared the effects of WL alone to a combined treatment of WL and aerobic exercise training on direct measures of insulin sensitivity. Only severe energy restriction in combination with aerobic exercise and light weight lifting but not WL alone improved glucose utilization in glucose intolerant and diabetic subjects (16). Similarly, in overweight men, glucose utilization increased after aerobic training alone or in combination with WL, whereas WL alone had no effect (18). Thus, a combination of exercise and WL may be necessary to elicit maximal improvements in insulin sensitivity. Moreover, potential mediating factors such as changes in visceral fat, intramuscular fat or FFA metabolism were not addressed. We hypothesized that the addition of aerobic training to a WL program would result in greater reductions in visceral and intramuscular fat than WL alone, resulting in greater improvements in *in vivo* glucose utilization. We also hypothesized that the rate of decline in FFA during hyperinsulinemia would increase after WL interventions and be associated with an improvement in glucose metabolism. Thus, the purpose of this study was to compare changes in visceral and intramuscular fat, insulin-stimulated glucose uptake, and the rate of decline in FFA concentrations during hyperinsulinemia in response to 6 months of WL or WL plus aerobic exercise (WL+AEX) in overweight and obese sedentary postmenopausal women.

Research Methods and Procedures

All subjects were healthy overweight or obese (BMI > 25 kg/m²; range, 25 to 41 kg/m²) women between the ages of 50 and 70 years. The women were postmenopausal and had not menstruated for at least 1 year and had plasma follicle-stimulating hormone levels >30 mIU/mL. Only women who were weight stable (<2.0 kg weight change in the past year) and sedentary (<20 minutes of aerobic exercise two times per week) were recruited. Subjects were screened by medical history questionnaire, physical exam-

ination, fasting blood profile, and a graded exercise treadmill test in an attempt to exclude those with cardiovascular disease. The women underwent a 2-hour 75-gram oral glucose tolerance test (OGTT) to exclude women with diabetes (19). All subjects were non-smokers and showed no evidence of cancer, liver, renal, or hematologic disease or other medical disorders.

Forty-seven women met all study criteria and were enrolled into the study and placed in either the WL ($n = 23$) or WL+AEX group ($n = 24$). Fourteen women dropped out of the program because of personal reasons, relocation, illness, and/or time constraints. Thus, 33 women completed the study and are included in this report. Sixteen women completed WL (11 white and 5 African American), and 17 women completed WL+AEX (14 white and 3 African American). Eight women ($n = 6$ in WL and $n = 2$ in WL+AEX) were using hormone replacement therapy for at least 3 years before enrollment, which did not change for the duration of the study. The Institutional Review Board of the University of Maryland approved all methods and procedures. Each participant provided written informed consent to participate in the study.

Weight Loss Program

During the 6-month WL intervention, all women in the interventions attended weekly WL classes led by a registered dietitian for instruction in the principles of a hypocaloric diet that followed the American Heart Association Step I (20) guidelines. Compliance was monitored by weekly review of 7-day food records and 24-hour dietary recalls during the 6 months. Women were instructed to restrict their caloric intake by 250 to 350 kcal/d. The program focused on eating behavior, stress management, control of portion sizes, and modification of binge eating. The average compliance with the WL classes was 80% for the WL group and 78% for the WL+AEX group.

Aerobic Exercise Program

Women in the WL+AEX intervention exercised at the Baltimore VA Medical Center and Geriatric Research, Education and Clinical Center exercise facility three times a week for 6 months using treadmills, cycle ergometers, and a track. Each exercise session included a 5- to 10-minute stretching and warm-up phase and a 5- to 10-minute cool-down phase. Women exercised at ~50% to 60% heart rate reserve and gradually progressed in duration and intensity until they were able to exercise at >60% maximum amount of oxygen (VO_{2max}) for 45 minutes. The average compliance with the exercise sessions was 78%.

VO_{2max} and Body Composition

VO_{2max} was measured using a continuous treadmill test protocol as previously described (7). Height (centimeters) and weight (kilograms) were measured to calculate BMI as

¹ Nonstandard abbreviations: FFA, free fatty acid; WL, weight loss; WL+AEX, weight loss plus aerobic exercise; OGTT, oral glucose tolerance test; VO_{2max} , maximum amount of oxygen; WHR, waist-to-hip ratio; FFM, fat-free mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; M, glucose utilization; I, plasma insulin; IRS-1, insulin receptor substrate-1.

weight (kilograms)/height (meters squared). 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). Fat mass, lean tissue mass, and bone mineral content were determined by DXA (model DPX-L; LUNAR Radiation Corp., Madison, WI) using the 1.3z DPX-L extended analysis program. Fat-free mass (FFM) is reported as lean tissue plus bone mineral content. A single 5-mm computed tomography scan was taken at the L₄-L₅ region using a General Electric Hi-Light Scanner to determine relative proportions of visceral adipose tissue (VAT) area, subcutaneous adipose tissue (SAT) area, and sagittal diameter (5). A second scan at the level of the mid-thigh was used to quantify muscle area, total fat area of the thigh, and low-density lean tissue of both the right and left legs as previously described (5).

Metabolic Testing

All subjects were weight stabilized (<1 kg) for at least 2 weeks before metabolic testing before and after the interventions. All subjects were provided with a eucaloric diet for 2 days before the clamp by a registered dietitian to control nutrient intake as previously described (7). All testing was performed in the morning after a 12-hour overnight fast. At the end of the 6-month program, the women in the WL+AEX group were asked to continue the aerobic training 3 d/wk during the final testing period, and the glucose clamps were performed 36 to 48 hours after the last bout of exercise.

Hyperinsulinemic-Euglycemic Clamps

Peripheral tissue sensitivity to exogenous insulin was measured using the hyperinsulinemic-euglycemic clamp technique (21). Difficulty in obtaining venous access occurred in three women (WL: $n = 2$, WL+AEX: $n = 1$), and the pre- and post-clamp was not performed. Arterialized blood was obtained from a dorsal heated hand vein (22). For the assessment of basal glucose and insulin levels, three arterialized blood samples were drawn at 10-minute intervals. Blood samples were obtained every 5 and 10 minutes thereafter for the determination of plasma glucose and insulin levels. A 10-minute priming and continuous infusion of insulin (240 pmol/m² per minute; Humulin, Eli Lilly Co., Indianapolis, IN) was performed for 180 minutes. A 20% glucose solution was used, which was measured as 18%. For the measurement of FFAs, blood was obtained at fasting, at 10-minute intervals during the first 60 minutes of the insulin infusion, and at 30-minute intervals thereafter until the 3 hours of insulin infusion had elapsed.

The mean plasma glucose level during 10 to 180 minutes 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 did not

differ before and after the interventions and averaged 5.36 ± 0.13 vs. 5.06 ± 0.13 mM for WL and 5.27 ± 0.10 vs. 4.90 ± 0.12 mM for WL+AEX. This was $97.6 \pm 0.1\%$ of the desired goal with a coefficient of variation of $5.0 \pm 0.2\%$ in all clamps ($n = 60$). Plasma insulin concentrations during 120 to 180 minutes of the hyperinsulinemic-euglycemic clamps were not different before or after the interventions (WL, 490 ± 24 vs. 456 ± 19 pM; WL+AEX, 473 ± 17 vs. 450 ± 10 pM; 467 ± 9 pM among all clamps, $n = 60$).

Indirect Calorimetry. Continuous indirect calorimetry was performed before the start of the glucose infusion and during the last 30 minutes of the insulin infusion by the open circuit dilution technique using a SensorMedics DeltaTrac cart (Yorba Linda, CA) to quantitate rates of glucose and fat oxidation with correction for protein oxidation based on 24-hour urinary urea nitrogen as previously described (7). Non-oxidative glucose metabolism is the difference between total glucose uptake and glucose oxidation.

Analysis of Blood Samples. Blood samples were collected in heparinized syringes and placed in pre-chilled test tubes containing 1.5 mg EDTA/mL of blood. The blood samples were centrifuged at 4 °C, and plasma was stored at -70 °C until analysis. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Insulin and leptin levels were determined by RIA (Linco, St. Louis, MO). Serum non-esterified fatty acids were quantitated by an acyl-CoA oxidase-based calorimetric kit (Wako Chemicals, Richmond, VA). Samples for glucose, insulin, leptin, and FFAs were measured in duplicate with a coefficient of variation of <10%, and the average of the two values was used in the statistical analyses.

Statistical Analyses

The computed tomography scans of the mid-thigh muscle area, total fat area, and low-density lean tissue area were not different between the right and left legs. Therefore, the values of the right leg were used in the statistical analyses for these outcomes. For the hyperinsulinemic-euglycemic clamps, the mean concentrations of glucose and insulin were calculated for each sample time-point. The trapezoidal rule was used to calculate the integrated response over 30-minute intervals from 30 to 180 minutes for each subject. The integrated response was divided by its time interval to compute mean concentrations. Glucose utilization (M) for 30-minute 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 (I), and was calculated by dividing the glucose utilized by the insulin concentration during the last 60 minutes of the clamp for each

Table 1. Physical characteristics of postmenopausal women before and after the interventions

	WL (<i>n</i> = 16)		WL+AEX (<i>n</i> = 17)	
	Before	After	Before	After
Age (years)	56 ± 1		59 ± 1	
Weight (kg)	88.8 ± 3.8	83.6 ± 3.7‡	85.8 ± 2.9	78.4 ± 2.6‡
Waist circumference (cm)	94.5 ± 2.7	91.0 ± 2.6†	92.7 ± 2.6	88.6 ± 2.4†
Hip circumference (cm)	119.4 ± 3.1	114.2 ± 3.1†	113.6 ± 2.5	110.0 ± 2.1†
WHR	0.79 ± 0.01	0.80 ± 0.02	0.82 ± 0.02	0.81 ± 0.01
Percent body fat	48.3 ± 0.9	45.6 ± 1.1‡	46.0 ± 1.5	42.5 ± 1.3‡
FM (kg)	42.7 ± 2.4	38.0 ± 2.5‡	39.1 ± 2.2	33.3 ± 1.8‡
FFM (kg)	44.9 ± 1.5	44.1 ± 1.3*	45.2 ± 1.5	44.3 ± 1.3
VO _{2max} (liters/min)	1.73 ± 0.10	1.63 ± 0.10*	1.73 ± 0.08	1.83 ± 0.08*

WL, weight loss; WL+AEX, weight loss plus aerobic exercise; WHR, waist-to-hip ratio; FM, fat mass; FFM, fat-free mass; VO_{2max}, maximum amount of oxygen. Values are means ± standard error.

Significantly different before vs. after the intervention: * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.005$.

subject. The metabolic clearance rate of insulin was calculated as described by Elahi et al. (23). The rate of disappearance of FFA concentrations during the clamp was also determined. The FFA concentration curve was plotted semi-logarithmically against the duration of the insulin infusion for each individual subject. The slope of the decline in FFA concentrations during the clamp was obtained by linear regression analysis from 0 to 50 minutes.

To compare the effects of the two treatment groups, differences in the change values (post value minus pre-value) were tested for significance using ANOVA with a dichotomous variable for group, hormone replacement therapy status, and the baseline value for each variable added to the model. Within-group differences between pre-intervention and post-intervention measures of variables were determined using a paired Student's *t* test. The two groups were combined, and univariate regression analyses were used to determine predictors of glucose utilization and insulin sensitivity. Stepwise regression analysis was used to determine whether changes in body composition and aerobic fitness were significant predictors of changes in glucose utilization and insulin sensitivity. Statistical significance was set at $p < 0.05$ for all tests. All data were analyzed by SPSS statistical software (SPSS, Chicago, IL). All values are expressed as mean ± standard error.

Results

Baseline Characteristics

The baseline physical characteristics of the women in each group are presented in Table 1. Age, body weight, waist and hip circumferences, WHR, and VO_{2max} were similar between groups at baseline. In addition, there were no

differences at baseline in percentage body fat, fat mass, FFM, VAT, SAT, sagittal diameter, mid-thigh muscle, mid-thigh subcutaneous fat, and mid-thigh low-density lean tissue area between groups (Table 2). Although fasting plasma glucose values were normal in all volunteers, one woman in the WL group and three women in the WL+AEX group had impaired glucose tolerance after OGTT. Fasting plasma glucose, insulin, leptin, and FFA concentrations and all other measures of glucose metabolism did not differ between groups at baseline (Tables 3 and 4).

Effects of WL

Body weight decreased 6% in the WL group ($p < 0.001$) along with significant decreases in waist and hip circumferences but no change in WHR (Table 1). VO_{2max} (liters per minute) also decreased 6% in the WL group ($p < 0.05$). The changes in body composition in the WL group showed an absolute decrease in percentage body fat of 3% ($p < 0.001$), a 12% decrease in total fat mass ($p < 0.001$), and a small but significant decrease (−1.6%) in FFM (Table 1; $p < 0.05$). With WL, there was an 18% decrease in VAT ($p < 0.001$), a 14% decrease in SAT ($p < 0.01$), a 9% decrease in sagittal diameter ($p < 0.001$), a 15% decrease in mid-thigh subcutaneous fat area ($p < 0.01$), and a 32% decrease in mid-thigh low-density lean tissue ($p < 0.05$), but no change in mid-thigh muscle area (Table 2).

Fasting plasma glucose and leptin concentrations decreased with WL (Table 3; $p < 0.05$), but fasting insulin and FFA concentrations did not change. Although basal carbohydrate oxidation did not change, basal fat oxidation decreased with WL ($p < 0.05$). Glucose utilization, insulin sensitivity, and oxidative and non-oxidative glucose disposal did not change after WL. Fat oxidation during insulin

Table 2. Abdominal and mid-thigh body composition of postmenopausal women before and after the interventions

	WL (n = 16)		WL+AEX (n = 17)	
	Before	After	Before	After
VAT area (cm ²)	140.4 ± 12.1	115.1 ± 11.5‡	143.6 ± 11.2	117.3 ± 8.7†
SAT area (cm ²)	497.7 ± 29.4	424.3 ± 31.9†	452.3 ± 28.8	371.8 ± 22.2‡
Sagittal diameter (mm)	26.4 ± 0.8	24.1 ± 0.8‡	25.4 ± 0.7	23.3 ± 0.5‡
Mid-thigh muscle area (cm ²)	75.2 ± 4.4	79.5 ± 3.1	81.0 ± 3.8	84.9 ± 5.3
Mid-thigh subcutaneous fat (cm ²)	217.1 ± 20.7	175.7 ± 14.6†	196.8 ± 14.3	142.3 ± 11.7‡
Mid-thigh low-density lean tissue (cm ²)	21.4 ± 2.9	14.6 ± 1.4*	22.1 ± 3.4	14.7 ± 1.5*

WL, weight loss; WL+AEX, weight loss plus aerobic exercise; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. Values are means ± standard error.

Significantly different before vs. after the intervention: * *p* < 0.05; † *p* < 0.01; ‡ *p* < 0.005.

stimulation decreased after WL (*p* < 0.05). The metabolic clearance rate of insulin was faster after WL (*p* < 0.05). FFA concentrations declined during hyperinsulinemia before and after WL (*p* < 0.001), reached a plateau at ~90 minutes, and showed a 93 ± 1% vs. 89 ± 2% suppression at 180 minutes before and after WL, respectively. The rate of disappearance of FFA concentration did not change with WL (1.02 ± 0.09 vs. 0.96 ± 0.09/h; Figure 1A).

Effects of WL+AEX

Body weight decreased 8% in the WL+AEX group (Table 1; *p* < 0.001). Waist and hip circumferences decreased similarly (*p* < 0.001); thus, there was no change in WHR. VO_{2max} increased 7% with WL+AEX (*p* < 0.05). There was an absolute decrease in percentage fat of 3% (*p* < 0.01), a 14% decrease in fat mass (*p* < 0.001), and no change in FFM (Table 1). VAT and SAT each decreased by

Table 3. Basal and clamp levels of various parameters of postmenopausal women before and after the interventions

	WL (n = 16)		WL+AEX (n = 17)	
	Before	After	Before	After
Basal				
Plasma glucose (mM)	5.4 ± 0.1	5.2 ± 0.1†	5.3 ± 0.1	5.1 ± 0.1†
Plasma insulin (pM)	63 ± 9	55 ± 5	55 ± 6	47 ± 4*
Plasma leptin (pM)	33.2 ± 3.3	28.3 ± 3.9*	26.4 ± 2.7	15.9 ± 1.8‡
Plasma FFA (mM)	1.14 ± 0.09	1.10 ± 0.11	1.02 ± 0.08	0.96 ± 0.07
Carbohydrate oxidation (μmol/kg _{FFM} /min)	8.2 ± 2.1	10.0 ± 1.4	9.4 ± 1.9	10.9 ± 2.3
Fat oxidation (μmol/kg _{FFM} /min)	8.9 ± 0.8	6.7 ± 0.6†	6.7 ± 0.6	5.7 ± 0.7
120 to 180 minutes of clamp				
Glucose utilization (μmol/kg _{FFM} /min)	47.8 ± 3.3	48.7 ± 4.3	55.7 ± 4.8	60.8 ± 4.2*
Non-oxidative glucose disposal (μmol/kg _{FFM} /min)	25.5 ± 2.5	29.5 ± 4.0	32.6 ± 3.3	36.9 ± 3.4
Oxidative glucose disposal (μmol/kg _{FFM} /min)	21.9 ± 2.5	20.7 ± 1.6	21.9 ± 2.5	24.7 ± 2.6
Fat oxidation (μmol/kg _{FFM} /min)	4.3 ± 0.9	2.8 ± 0.7*	2.8 ± 0.5	1.4 ± 0.4*
Insulin sensitivity (μmol/kg _{FFM} /min/pM)	0.102 ± 0.009	0.111 ± 0.013	0.122 ± 0.012	0.136 ± 0.009*
Insulin clearance (mL/m ² /min)	496.6 ± 21.3	534.1 ± 23.7*	533.6 ± 12.6	512.9 ± 18.1

WL, weight loss; WL+AEX, weight loss plus aerobic exercise; FFA, free fatty acid. Values are means ± standard error.

Significantly different before vs. after the intervention: * *p* < 0.05; † *p* < 0.01; ‡ *p* < 0.005.

Table 4. Predictors of the change in glucose utilization and insulin sensitivity in women

Pearson correlation coefficients	ΔM	$\Delta M/I$
ΔVO_2	0.15	0.07
Δ Fat mass	-0.40*	-0.48†
Δ VAT	-0.48†	-0.56†
Δ SAT	-0.30	-0.51†
Δ Mid-thigh intramuscular fat area	-0.04	-0.08
Δ Rate of FFA disappearance	-0.35‡	-0.31

M, glucose utilization; M/I, amount of glucose metabolized per unit of plasma insulin; VO_2 , maximum amount of oxygen; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; FFA, free fatty acid.

* $p < 0.05$; † $p < 0.01$; ‡ $p = 0.06$.

17% ($p < 0.01$), sagittal diameter decreased by 8% ($p < 0.001$), mid-thigh subcutaneous fat decreased by 27% ($p < 0.001$), and mid-thigh intramuscular fat decreased by 18% (Table 2; $p < 0.05$). There was no change in mid-thigh muscle area after WL+AEX.

Fasting plasma glucose, insulin, and leptin concentrations decreased after WL+AEX (Table 3; $p < 0.05$), but fasting FFA concentrations did not change. Basal carbohydrate and fat oxidation did not change with WL+AEX. Moreover, glucose utilization increased 15% ($p < 0.05$) and insulin sensitivity increased 21% ($p < 0.05$). Although oxidative glucose disposal did not change, there was a trend for non-oxidative glucose metabolism to increase ($p = 0.07$). Fat oxidation during the clamp decreased after WL+AEX ($p < 0.05$). Insulin clearance rate did not change after WL+AEX. FFA concentrations decreased during the insulin infusion before and after WL+AEX ($p < 0.001$), reached a plateau at ~90 minutes, and showed a 92 ± 2% vs. 93 ± 1% suppression at 180 minutes before and after WL+AEX, respectively (Figure 1B). However, the rate of disappearance of FFA concentrations did not change after WL+AEX (1.13 ± 0.12 vs. 1.14 ± 0.10/h).

Group Comparisons

There were no differences in changes in body composition between the two groups. However, changes in VO_{2max} were significantly different between the WL and WL+AEX groups ($p < 0.05$). In general, there were no differences in changes in metabolism between groups, except changes in plasma leptin concentrations were greater in the WL+AEX group ($p = 0.06$) than WL alone.

Predictors of Changes in Glucose Utilization and Insulin Sensitivity

At baseline, VAT and intramuscular fat were inversely related to M ($r = -0.44$ and $r = -0.41$, $p < 0.05$) and M/I

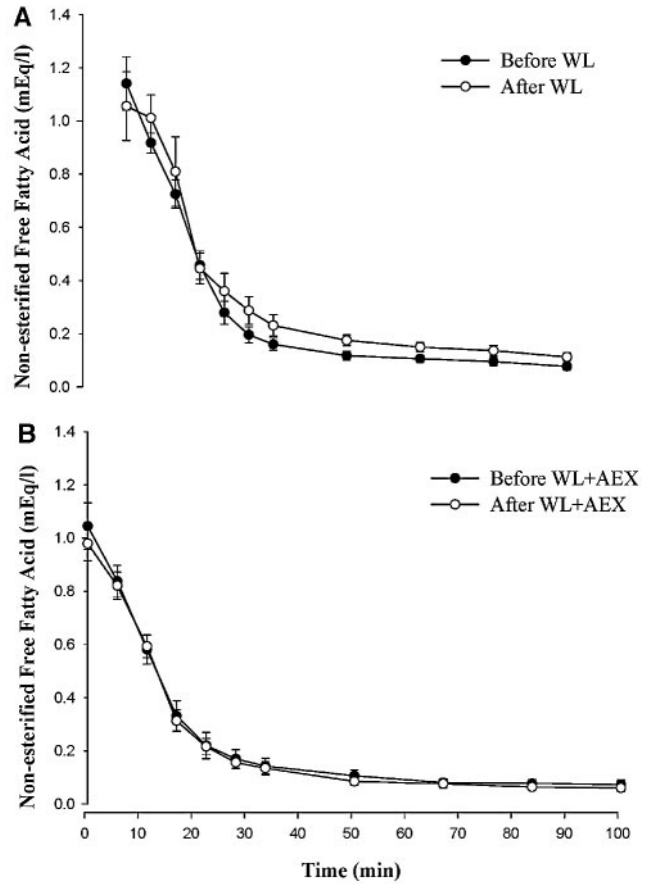


Figure 1: The reduction in FFA levels in response to insulin infusion before and after 6 months of weight loss (A) and WL+AEX (B) in obese postmenopausal women.

($r = -0.42$ and $r = -0.43$, $p < 0.05$). The rate of FFA disappearance was also inversely associated with M ($r = -0.75$, $p < 0.01$) and M/I ($r = -0.77$, $p < 0.001$). Changes in M were indirectly related to changes in fat mass and VAT (Figure 2; $p < 0.05$) and the rate of FFA disappearance ($p = 0.06$; Table 4). Changes in M were not related to changes in VO_{2max} or mid-thigh intramuscular fat. The predictors of changes in M/I included changes in total fat mass, VAT, and SAT (Table 4). Changes in these variables (VO_{2max} , total fat mass, VAT, SAT) were entered into a stepwise regression model to determine the independent predictors of changes in M and M/I. Changes in VAT independently predicted improvements in glucose utilization ($r^2 = 0.30$, $p < 0.05$) and insulin sensitivity ($r^2 = 0.33$, $p < 0.05$).

Discussion

The results of this study indicate that a 6-month program of WL alone and WL combined with aerobic exercise training are equally effective in reducing body weight, visceral fat, and intramuscular fat in obese postmenopausal

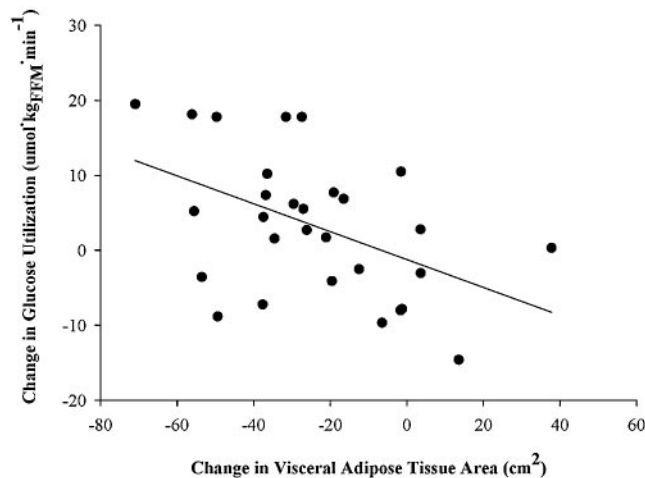


Figure 2: The relationship between the change in glucose utilization to the change in VAT with the WL interventions in obese postmenopausal women ($r = -0.48$, $p < 0.01$).

women. However, only WL+AEX resulted in significant improvements in glucose utilization and insulin sensitivity. Moreover, in the entire group, increases in glucose utilization were associated with reductions in visceral fat.

Previous studies report that low-calorie dieting reduces hyperinsulinemia and increases glucose utilization in obese men and premenopausal women (13,24). Very-low-calorie dieting is also successful in reducing hyperinsulinemia, improving glycemic control and increasing glucose disposal in patients with type 2 diabetes (25,26). However, in our postmenopausal women, no overall changes in glucose metabolism were observed with a moderate caloric restriction WL program. It is possible that significant changes would have resulted if we had enrolled only women with either impaired glucose tolerance or type 2 diabetes or if there had been greater losses of body weight. Our results are similar to those observed in obese older men where glucose utilization increased significantly after aerobic exercise alone and when combined with WL, but did not change after WL alone (18). This is also consistent with reported 2-fold greater reductions in glucose and insulin areas after diet plus aerobic exercise than diet alone in obese men (27). We are not aware of any studies in postmenopausal women that compared WL and WL+AEX effects on in vivo glucose metabolism. In this study, insulin sensitivity increased 21% after WL+AEX compared with a lack of significant change in insulin action after WL alone. This suggests that the addition of exercise training to moderate WL is critical to improve glucose metabolism in obese postmenopausal women.

In postmenopausal women, visceral fat is a significant predictor of insulin-stimulated glucose uptake (7). In addition, the loss of visceral fat is the best predictor of the improvement in insulin sensitivity after WL alone in young

subjects (13). However, this contrasts with a recent study where similar losses of abdominal and intermuscular fat were observed after 3 months of diet alone and diet combined with aerobic exercise but changes in glucose and insulin OGTT areas were not related to changes in any of the body fat variables (28). Perhaps this discrepancy is caused by the difference in methodology for assessing glucose homeostasis. In this study, the reduction in VAT was the best independent predictor of the improvement in glucose utilization and insulin sensitivity in the entire sample regardless of exercise status. Thus, our results confirm those results reported in young individuals after WL (13) and adds that losses of visceral fat predict improvements in glucose metabolism with WL alone and combined with aerobic training in older women.

Skeletal muscle triglyceride is increased in obesity and associated with insulin resistance whether it is determined by reduced muscle attenuation with computed tomography (5–7), histological sections with staining for triglyceride content of the myocytes (29), or by extraction and isolation of muscle lipid content (30). Intramyocellular lipid accumulation is associated with insulin resistance through impairments in skeletal muscle insulin signaling, specifically reductions in insulin stimulation of tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) associated phosphatidylinositol 3-kinase activity (9). WL, alone (13,14) or with low-intensity walking (15), decreases the lipid stored in skeletal muscle in obese men and women. We show in this study that WL and WL+AEX result in a similar loss of intramuscular fat. It seems that weight reduction per se is important in the loss of intramuscular fat and not the addition of exercise training. Although glucose utilization and insulin sensitivity were inversely associated with intramuscular fat at baseline, and the decrease of intramuscular fat was similar to the loss in visceral fat with the interventions, changes in insulin sensitivity were not related to changes in intramuscular fat.

In this study, we found a decrease in resting fat oxidation with WL, but no change in resting fat oxidation when aerobic training was added to the WL program. Our results are in agreement with those reported in obese women (31) that the addition of exercise training to energy restriction counteracts the decline in fat oxidation with WL alone. This could impact future weight maintenance because a low rate of fat oxidation is a risk factor for weight regain (32). WL alone results in a lower rate of lipid oxidation by indirect calorimetry (33) and lipid oxidation across leg tissues (34) during glucose clamps in young obese subjects. To the best of our knowledge, there is a lack of studies in the literature that examined lipid oxidation after WL+AEX. We observed a significant decrease in fatty acid oxidation during hyperinsulinemia before and after the interventions. Moreover, the rate of fat oxidation during hyperinsulinemia decreased after WL and WL+AEX.

Basal FFA levels did not change after WL or WL+AEX in these postmenopausal women. An elevation of fasting FFA levels impairs insulin-mediated vasodilation and nitric oxide production (13) and increases insulin resistance (10). Increased total body fat and visceral adiposity are associated with increased lipolysis and resistance to suppression by insulin (8). Furthermore, the rate of decline in FFA levels from basal during hyperinsulinemic-euglycemic clamps is delayed in obese men and associated with a reduction in glucose uptake (12). We confirm this in obese women, such that women with higher rates of disappearance of FFA had higher rates of insulin-stimulated glucose uptake. The ability of insulin to decrease circulating FFA concentrations is impaired in healthy men with high intramyocellular lipid content (9). Boden et al. (10) showed that the decrease in FFA levels during clamps is associated with a decrease in intramyocellular fat content by proton nuclear magnetic resonance spectroscopy in young healthy subjects and suggested that accumulation of triglycerides within muscle fibers is an important step in FFA-induced insulin resistance. Previous reports indicate that there is an improved insulin suppression of lipolysis and lipid oxidation after WL in young obese subjects (35). Although we are unaware of any studies that examined changes in insulin's anti-lipolytic effect in vivo after WL with exercise training in postmenopausal women, we hypothesized that the rate of decline in FFA levels in response to hyperinsulinemia would increase after WL and exercise. Our results indicate that neither the ability of insulin to eventually suppress FFA concentrations from basal to steady state during the clamp nor the rate of disappearance of FFA concentration changes after WL or WL+AEX. However, the change in the disappearance of FFA levels tended to be associated with the change in M, suggesting that women who were able to increase the rate of FFA decline tended to have the greatest improvement in glucose utilization and that these women became more sensitive to insulin's anti-lipolytic effect. Although we did not study the cellular mechanisms for FFA influence on glucose metabolism, Griffin (36) showed a decrease in rat skeletal muscle IRS-1 associated PI 3-kinase activity and a blunting in insulin-stimulated IRS-1 phosphorylation during glucose clamps with concomitant lipid/heparin infusions. Their results suggest that, in the rat model, fatty acids or their metabolites inhibit insulin signaling at points preceding activation of glycogen synthase or glucose transport. Future studies could be directed toward assessing the role of fatty acids and triglycerides stored in skeletal muscle in the insulin-stimulated state in obese individuals and potential changes after WL and exercise training.

There are several limitations to this study. Our sample size may have been inadequate to detect a treatment difference in glucose utilization. Our power calculations showed that, given our sample size, we had 35% power to detect a one-sided difference. At 80% power and $\alpha = 0.05$, at least

57 women per group would be necessary to detect a statistically significant difference in glucose utilization using a one-sided test. In addition, we did not measure the rate of hepatic glucose production. Although we believe that hepatic glucose production was completely suppressed before and after the interventions, it is possible that hepatic insulin sensitivity was altered with the WL interventions.

We conclude that 6 months of WL+AEX, but not WL alone, results in significant increases in glucose utilization and insulin sensitivity in obese postmenopausal women. Furthermore, improvements in glucose metabolism were associated with reductions in visceral fat, suggesting that losses of abdominal fat may mediate changes in insulin resistance.

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References

1. **Kuczmarski RJ, Carroll MD, Flegal KM, Troiano RP.** Varying body mass index cutoff points to describe overweight prevalence among US adults. *NHANES II (1988–1994). Obes Res.* 1997;5:542–8.
2. **Poehlman ET, Toth MJ, Gardner AW.** Changes in energy balance and body composition at menopause: a controlled longitudinal study. *Ann Intern Med.* 1995;123:673–5.
3. **Serdula AG, Mokdad AH, Williamson DF, Galuska DA, Menlein JM, Heath GW.** Prevalence of attempting weight loss and strategies for controlling weight. *JAMA.* 1999;282:1353–8.
4. **Rafferty AP, Reeves MJ, McGee HB, Pivarnik JM.** Physical activity patterns among walkers and compliance with public health recommendations. *Med Sci Sports Exerc.* 2002;34:1255–61.
5. **Ryan AS, Nicklas BJ.** Age-related changes in fat deposition in mid-thigh muscle in women: relationships with metabolic cardiovascular disease risk factors. *Int J Obes Relat Metab Disord.* 1999;23:126–32.
6. **Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE.** Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes.* 1997;46:1579–85.
7. **Ryan AS, Nicklas BJ, Berman DM.** Insulin resistance and fat deposition within mid-thigh muscle differ between obese African American and Caucasian postmenopausal women. *Obes Res.* 2002;10:336–44.

8. **Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM.** Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest.* 1989;83:1168–73.
9. **Virkkamaki A, Korshennikova E, Seppala-Lindroos A, et al.** Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. *Diabetes.* 2001;50:2337–43.
10. **Boden G, Lebed B, Schatz M, Homko C, Lemieux S.** Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes.* 2001;50:1612–7.
11. **Roden M, Price TB, Pereghin G, et al.** Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest.* 1996;97:2859–65.
12. **Coon PJ, Rogus EM, Goldberg AP.** Time course of plasma free fatty acid concentration in response to insulin. Effect of obesity and physical fitness. *Metabolism.* 1992;41:711–6.
13. **Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL.** Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes.* 1999;48:839–47.
14. **Greco AV, Mingrone G, Giancaterini A, et al.** Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes.* 2002;51:144–51.
15. **Ryan AS, Nicklas BJ, Berman DM, Dennis KE.** Dietary restriction and walking reduce fat deposition in the mid-thigh in obese older women. *Am J Clin Nutr.* 2000;72:708–13.
16. **Bogardus C, Ravussin E, Robbins DC, Wolfe RR, Horton ES, Sims EA.** Effects of physical training and diet therapy on carbohydrate metabolism in patients with glucose intolerance and non-insulin dependent diabetes mellitus. *Diabetes.* 1984;33:311–8.
17. **Hughes VA, Fiatarone MA, Fielding RA, et al.** Exercise increases muscle GLUT4 levels and insulin action in subjects with impaired glucose tolerance. *Am J Physiol.* 1993;264:E855–62.
18. **Dengel DR, Pratley RE, Hagberg JM, Rogus EM, Goldberg AP.** Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. *J Appl Physiol.* 1996;81:318–25.
19. **American Diabetes Association and National Institute Diabetes Digestive and Kidney Disease.** The prevention or delay of type 2 diabetes. *Diabetes Care.* 2002;25:742–9.
20. **American Heart Association Steering Committee.** Dietary guidelines for healthy American adults. *Circulation.* 1988;77:721–4.
21. **DeFronzo RA, Tobin JD, Andres R.** Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214–33.
22. **McGuire EAH, Helderman JH, Tobin JD, Andres R, Berman M.** Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol.* 1976;41:565–73.
23. **Elahi D, Nagulesparan M, Hershcopf RJ, et al.** Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N Engl J Med.* 1982;306:1196–202.
24. **Ross R, Dagnone D, Jones PJ, et al.** Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. *Ann Intern Med.* 2000;133:92–103.
25. **Wing RR, Blair E, Marcus M, Epstein LH, Harvey J.** Year-long weight loss treatment for obese patients with type II diabetes: does including an intermittent very-low-calorie diet improve outcome? *Am J Med.* 1994;97:354–62.
26. **Henry RR, Wallace P, Olefsky JM.** Effects of weight loss on mechanisms of hyperglycemia in obese non-insulin-dependent diabetes mellitus. *Diabetes.* 1986;35:990–8.
27. **Rice B, Janssen I, Hudson R, Ross R.** Effects of aerobic or resistance exercise and/or diet on glucose tolerance and plasma insulin levels in obese men. *Diabetes Care.* 1999;22:684–91.
28. **Janssen I, Fortier A, Hudson R, Ross R.** Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care.* 2002;25:431–8.
29. **Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R.** Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol.* 2000;89:104–10.
30. **Pan DA, Lillioja S, Kriketos AD, et al.** Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* 1997;46:983–8.
31. **Nicklas BJ, Rogus EM, Goldberg AP.** Exercise blunts declines in adipocyte lipolysis and fat oxidation after dietary-induced weight loss in obese, postmenopausal women. *Am J Physiol.* 1997;273:E149–55.
32. **Zurlo F, Lillioja S, Esposito-Del Puente A, et al.** Low ratio of fat to carbohydrate oxidation as a predictor of weight gain: a study of 24-hr RQ. *Am J Physiol.* 1990;259:E650–7.
33. **Ranneries C, Bulow J, Buemann B, Christensen NJ, Madsen J, Astrup A.** Fat metabolism in formerly obese women. *Am J Physiol.* 1998;274:E155–61.
34. **Kelly DE, Goodpaster B, Wing RR, Simoneau J.** Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol.* 1999;277:E1130–41.
35. **Bryson JM, King SE, Burns CM, Baur LA, Swaraj S, Catterton ID.** Changes in glucose and lipid metabolism following weight loss produced by a very low calorie diet in obese subjects. *Int J Obes Relat Metab Disord.* 1996;20:338–45.
36. **Griffin ME, Marcucci MJ, Cline GW, et al.** Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes.* 1999;48:1270–4.