Effects of Weight Loss on Regional Fat Distribution and Insulin Sensitivity in Obesity

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Weight loss (WL) decreases regional depots of adipose tissue and improves insulin sensitivity, two parameters that correlate before WL. To examine the potential relation of WL-induced change in regional adiposity to improvement in insulin sensitivity, 32 obese sedentary women and men completed a 4-month WL program and had repeat determinations of body composition (dual-energy X-ray absorptiometry and computed tomography) and insulin sensitivity (euglycemic insulin infusion). There were 15 lean men and women who served as control subjects. Vo_{2max} was unaltered with WL (39.2 ± 0.8 vs. 39.8 ± 1.1 ml · fat-free mass [FFM]$^{-1}$ · min^{-1}). The WL intervention achieved significant decreases in weight (100.2 ± 2.6 to 85.5 ± 2.1 kg), BMI (34.3 ± 0.6 to 29.3 ± 0.6 kg/m^2), total fat mass (FM) (36.9 ± 1.5 to 26.1 ± 1.3 kg), percent body fat (37.7 ± 1.3 to 31.0 ± 1.5%), and FFM (59.2 ± 2.3 to 55.8 ± 2.0 kg). Abdominal subcutaneous and visceral adipose tissue (SAT and VAT) were reduced (494 ± 19 to 357 ± 18 cm^2 and 157 ± 12 to 96 ± 7 cm^2, respectively). Cross-sectional area of low-density muscle (LDM) at the mid-thigh decreased from 67 ± 5 to 55 ± 4 cm^2 after WL. Insulin sensitivity improved from 5.9 ± 0.4 to 7.3 ± 0.5 mg · FFM^{-1} · min^{-1} with WL. Rates of insulin-stimulated nonoxidative glucose disposal accounted for the majority of this improvement (3.00 ± 0.3 to. 4.3 ± 0.4 mg · FFM^{-1} · min^{-1}). Serum leptin, triglycerides, cholesterol, and insulin all decreased after WL (P < 0.01). After WL, insulin sensitivity continued to correlate with generalized and regional adiposity but, with the exception of the percent decrease in VAT, the magnitude of improvement in insulin sensitivity was not predicted by the various changes in body composition. These interventional weight loss data underscore the potential importance of visceral adiposity in relation to insulin resistance and otherwise suggest that above a certain threshold of weight loss, improvement in insulin sensitivity does not bear a linear relationship to the magnitude of weight loss. *Diabetes* 48:839–847, 1999

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CT, computed tomography; DEXA, dual-energy X-ray absorptiometry; ECG, electrocardiogram; FFM, fat-free mass; FM, fat mass; HPLC, high-performance liquid chromatography; HU, Hounsfield unit; LDM, low-density muscle; NDM, normal-density muscle; R_d, glucose utilization; ROI, region of interest; SAT, subcutaneous adipose tissue; TAT, thigh adipose tissue; VAT, visceral adipose tissue; VLCD, very-low-calorie diet; WL, weight loss.

Weight loss (WL) can have a potent effect to improve insulin sensitivity in overweight individuals (1–5), but whether this effect is due to reduction of a specific fat depot or generalized reduction of adipose tissue has not been clearly determined. A number of cross-sectional studies, based on data from lean and obese individuals, have found insulin resistance related more strongly to abdominal adiposity than to total adiposity (6–10). These results raise the question whether WL-induced improvement in insulin sensitivity is more strongly related to reductions in abdominal adiposity or to overall adiposity. It has been clearly established that WL can reduce visceral adiposity and fat mass (11–14) and improve insulin sensitivity of skeletal muscle (4,5). Intervention-based studies of weight loss, body composition, and insulin sensitivity would be a potentially useful adjunct to prior cross-sectional studies. Therefore, the current study was undertaken to measure total and regional patterns of adiposity in obese men and women before and after a dietary-induced weight loss. Fujioka et al. (15) did find that reductions in visceral adiposity achieved by weight loss were significantly associated with the improvement in oral glucose tolerance even after adjusting for overall weight loss. To our knowledge, however, no previous clinical investigation has assayed both changes in body composition and changes in insulin sensitivity. The current study was undertaken for this purpose.

Another aspect of regional fat distribution related to insulin resistance is lipid content within skeletal muscle (16,17). Consistent with the potential importance of lipid content in skeletal muscle for insulin resistance, our laboratory has recently reported that a reduced attenuation value of skeletal muscle in obesity, as determined by computed tomography and most likely reflecting an increased skeletal muscle fat content (18), is a strong marker of insulin resistance in obesity (19). However, the effects of WL on skeletal muscle composition in human obesity have not been well described, and relatively little is known concerning whether weight loss changes muscle lipid content and whether such changes might relate to changes in insulin sensitivity.

RESEARCH DESIGN AND METHODS

Subjects. Fifty-three healthy glucose-tolerant men and premenopausal women responding to public advertisement were selected to participate in this investigation. Eighteen obese men and 20 obese women, all with BMIs ≥30 kg/m^2, enrolled in a 16-week caloric restriction–induced weight loss program described below, while 7 men and 8 women, all with BMIs ≤27 kg/m^2, served as lean control subjects. Seventeen women and 15 men ages 38.7 ± 1.0 years successfully completed the WL program. Five of the 15 lean control subjects and 3 individuals participating in the weight loss program were African-Americans; the remaining volunteers were Caucasian. Individuals with diabetes, hyperlipidemia (plasma triglycerides ≥350 mg/dl or total

cholesterol levels ≥300 mg/dl), coronary heart disease, or vascular disease were excluded. Individuals with hypertension or being treated with antihypertensive agents were excluded. None of the volunteers participated in any regular exercise. The protocol was approved by the University of Pittsburgh Institutional Review Board, and all volunteers gave written informed consent.

Caloric restriction-induced weight loss. Volunteers completed a 16-week supervised weight loss program designed to achieve a 15-kg WL. For the first 10 weeks, subjects consumed a very-low-calorie diet (VLCD) (800 kcal/day) consisting of liquid formula (Optifast) and lean meat, fish, and fowl. During weeks 11–13, subjects consumed 1,200 kcal/day with a gradual reintroduction of fruits, vegetables, and grains to the diet. During weeks 14–16, subjects consumed a weight-maintaining diet, with 30% of calories from fat, 15% from protein, and 55% from carbohydrates. Post-WL assessments were performed at week 17 to avoid the confounding effects of acute fasting or caloric restriction. Serum potassium, uric acid, and electrocardiograms (ECGs) were measured after 2 weeks and then at 4-week intervals of the VLCD. Vitamin and mineral supplementation was provided during the VLCD.

Maximal aerobic capacity. To assess physical fitness before and after weight loss, maximal aerobic power (VO_{2max}) was measured using an incremental protocol on an electronically braked cycle ergometer (Bosch ERG 601, Stuttgart, Germany). Briefly, men and women began exercising for 2 min at 100 and 50 W, respectively, after which time resistance was increased 25 W every 2 min until volitional fatigue. Heart rate, blood pressure, and ECG were recorded before, during, and immediately after this test. During exercise, expired air was collected via a mouthpiece and two-way breathing valve into a 5-liter mixing chamber (Rayfield RMC-1, Waitsfield, VT) containing a bidirectional turbine to measure expiratory flow. A mass spectrometer (Marquette Electronics, Milwaukee, WI) was used to analyze expired air for CO_2 and O_2 fractions, and a computer analyzed and integrated signals for the determination of oxygen consumption (Vo_2) every 30 s.

Body composition

Dual energy X-ray absorptiometry. Whole-body fat mass (FM) and fat-free mass (FFM) were assessed by dual energy X-ray absorptiometry (DEXA) (model DPX-L; Lunar, Madison, WI) using software version 1.3Z. This computerized analysis was also employed to measure abdominal FM and fat mass and lean tissue mass in the mid-thigh region. Abdominal adipose tissue content was assessed by manually drawing a region of interest (ROI) on the abdomen, using the diaphragm as the upper (cephalad) limit and the top of the iliac spine as the lower (caudal) limit (20). Care was taken to position all volunteers with their arms separated from their trunks to include only abdominal areas in the analysis. Abdominal adipose tissue content (g) measured by DEXA was highly correlated to total abdominal adipose tissue area (cm^2) measured by computed tomography ($r = 0.97$, $P < 0.01$). Mid-thigh lean tissue and FM were measured by manually placing an ROI centered at the midpoint between the superior edge of the femur and the superior edge of the patella, spanning the width of both thighs at a height of 10 cm. Mid-thigh lean and adipose tissue content measured with DEXA was highly correlated to computed tomography (CT)-determined thigh muscle and thigh adipose tissue areas ($r = 0.95$, $P < 0.01$, and $r = 0.93$, $P < 0.01$, respectively).

Computed tomography. CT (9800 CT scanner; General Electric, Milwaukee, WI) was used to measure cross-sectional abdominal visceral and subcutaneous adipose tissue (VAT and SAT) areas using an established method (21). Briefly, a cross-sectional scan at 10-mm thickness was obtained, centered at the L4-L5 vertebral disc space using 170 mÅ with a scanning time of 2 s and a 512 × 512 matrix. The visceral and subcutaneous AT boundary was defined using a manual cursor, and adipose tissue areas were determined using commercially available software (General Electric). CT was also used to measure cross-sectional area of mid-thigh bone, muscle, and adipose tissue and to characterize muscle attenuation. With the subject supine, a 10-mm cross-sectional scan of both legs was obtained, located at the midpoint between the anterior iliac crest and the patella. In image analysis, areas of bone, adipose tissue, and skeletal muscle were measured by selecting the following ROIs defined by attenuation values: ≥200 Hounsfield units (HU) for bone, –30 to –190 HU for adipose tissue, and 0 to 100 HU for muscle; mean muscle attenuation was determined from all pixels within this range. Muscle area was further characterized as normal-density muscle (NDM) (cross-sectional area of muscle displaying attenuation values within 2 SDs of the mean attenuation value of lean, normal muscle) (31 to 100 HU), and low-density muscle (LDM) (muscle with lower than normal attenuation values) (0 to 30 HU).

Insulin sensitivity. Subjects were instructed to consume a weight-maintaining diet containing at least 200 g carbohydrate for at least 3 days before measurements of insulin sensitivity and to avoid exercise or strenuous exertion for 2 days before the studies. On the day before measurement of insulin sensitivity, subjects were admitted to the University of Pittsburgh General Clinical Research Center. That evening, they received a standard dinner (10 kcal/kg; 50% carbohydrate, 30% fat, 20% protein) and then fasted until completion of the insulin sensitivity measurement. An overnight, timed urine collection (~12 h duration) was obtained for nitrogen measurements to estimate protein oxidation with systemic indirect calorimetry. At ~7:00 A.M., a catheter was placed in a forearm vein for later infusion of glucose and insulin and to start a primed (20 µCi), continuous (0.20 µCi/min) infusion of high-performance liquid chromatography (HPLC)-purified [3-^{3}H]glucose (New England Nuclear, Boston, MA). Isotope was started 100 min before beginning the euglycemic insulin infusion and was given to determine glucose utilization during the final 30 min of the 3-h insulin infusion, thus allowing 4 h for isotope equilibration. An additional catheter was inserted into a radial artery for blood sampling. Baseline postabsorptive arterial samples were collected for determination of serum insulin, cholesterol, triglyceride, and leptin. Continuous infusion of regular insulin (Humulin; Eli Lilly, Indianapolis, IN) was given at a rate of 40 mU · m^{-2} · min^{-1} for 3 h, and euglycemia was maintained using an adjustable infusion of 20% dextrose, to which 80 µCi of [3-^{3}H]glucose was added to maintain stable plasma glucose specific activity (16). Plasma glucose was determined at 5-min intervals during the clamp. Blood samples for measurement of [3-^{3}H]glucose specific activity were collected every 10 min during the final 40 min of insulin infusion. Systemic indirect calorimetry was performed in the postabsorptive state and during the last 30 min of insulin infusion, using an open circuit spirometry metabolic monitor system (DeltaTrac, Anaheim, CA), to estimate glucose and fat oxidation.

Data analyses. Plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 Glucose Analyzer; YSI, Yellow Springs, OH). Glucose specific activity was determined with liquid scintillation spectrometry after the deproteinization of plasma with barium sulfate and zinc hydroxide. Serum insulin and leptin were determined using commercially available radioimmunoassay kits (Pharmacia, Uppsala, Sweden; Linco Research, St. Louis, MO). Serum HDL cholesterol, total cholesterol, and triglycerides were determined using a spectrophotometric assay.

Calculations. Rate of plasma glucose appearance (R_a) and utilization (R_d) were calculated using the Steele equations (22), as modified for variable-rate glucose infusions that contain isotope (23). Nonoxidative glucose disposal ($G_{non\text{-}ox}$) was calculated as the difference between R_d and glucose oxidation (G_{ox}). Rates of G_{ox} were calculated based on rates of gas exchange from indirect calorimetry (24).

Statistical analysis. Data are presented as mean ± SE unless otherwise indicated. Weight loss changes were compared using paired t tests. Analysis of variance was used to compare men and women for clinical, metabolic, body composition, and physical fitness parameters. Regression analysis was used to determine the relationships between changes in body composition and changes in insulin sensitivity. All statistics were performed using JMP version 3.1.6 for the Macintosh (SAS, Cary, NC).

RESULTS

Weight loss program. Thirty-eight obese individuals with BMI ≥30 kg/m^2 entered and 32 completed the 4-month WL program and post-WL assessments of body composition and insulin sensitivity. Of the three men and three women who did not complete the WL program, five discontinued during the initial 2 months of WL intervention; one individual completed the weight loss but not post-WL assessments. Mean weight loss of those subjects who did complete was 15.1 ± 1.2 kg, representing a 15 ± 0.9% decrease of body weight. As shown in Fig. 1, weight loss occurred rapidly at the start of the WL program and was progressive during the initial 12 weeks and then, as was intended by study design, weight was maintained at stable levels for 4 weeks leading into post-WL metabolic assessments.

Changes in body composition

Changes in FM and FFM. Mean BMI decreased from 34.5 ± 0.8 to 29.3 ± 0.8 kg/m^2; thus, despite substantial weight loss, most subjects remained obese, and just 5 of 17 women and 4 of 15 men attained a BMI ≤27 kg/m^2. There were sex-related differences in the amount of weight loss, expressed both in absolute weight loss and as percentage of initial weight (both $P < 0.05$). Among the 15 men, the mean weight loss was 17.5 ± 3.0 kg (15.8 ± 1.5% of initial weight), and among the 17 women, the mean weight loss was 12.7 ± 2.9 kg (13.3 ± 1.0% of initial weight). The principal effect of WL was a reduction in FM, and there was a smaller yet statistically significant loss of FFM, as shown in Fig. 2. Men lost more FFM than did women (4.6 vs. 2.1 kg, $P < 0.05$). Table 1 shows that men and women lost similar amounts of FM, with a mean FM loss of 11.3 ± 0.9 kg, which represented a reduction in FM of 29 ± 2%.

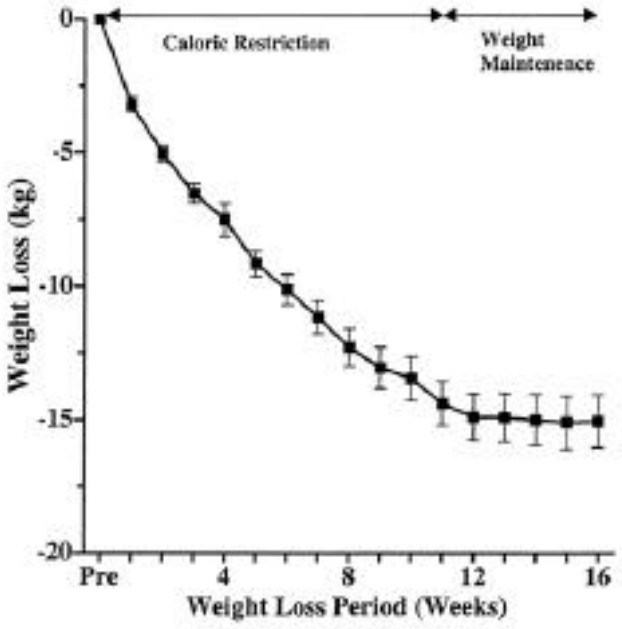

FIG. 1. Progression of WL during 12 weeks of caloric restriction and the 4-week weight stabilization phase before the post-WL metabolic and body composition assessments.

Changes in abdominal adipose tissue. There was substantial reduction in abdominal adipose tissue (Fig. 2). VAT, which before weight loss accounted for approximately one-quarter of cross-sectional abdominal adipose tissue (22 ± 1.9 and 26 ± 1.4% in women and men, respectively) decreased by 78 ± 10 cm^2 (44 ± 4%) in men and 44 ± 11 cm^2 (30 ± 3%) in women (Table 2). In post-WL men, despite not having attained a nonobese BMI, the mean value for VAT was similar to that found in the lean male control subjects. In post-WL women, however, values for VAT remained twofold greater than in lean female control subjects. There was also substantial loss of SAT, with decreases of 159 ± 15 and 118 ± 14 cm^2 in men and women, respectively, representing decreases of 34 ± 3 and 23 ± 2%. The loss of abdominal adipose tissue content determined with DEXA was 2.8 ± 0.3 kg (32 ± 3.7%) in women and 3.1 ± 0.4 kg (46 ± 6%) in men. Moreover, the loss of total abdominal fat measured with CT was significantly related to the loss of abdominal fat measured by DEXA ($r = 0.58$, $P < 0.01$).

Changes in thigh adipose tissue and muscle composition. Computed tomography was also employed to assess the impact of WL on mid-thigh skeletal muscle and mid-thigh adipose tissue (TAT). At baseline, obese men and women had greater TAT and skeletal muscle areas than lean men and women. Obese women had significantly more ($P < 0.05$)

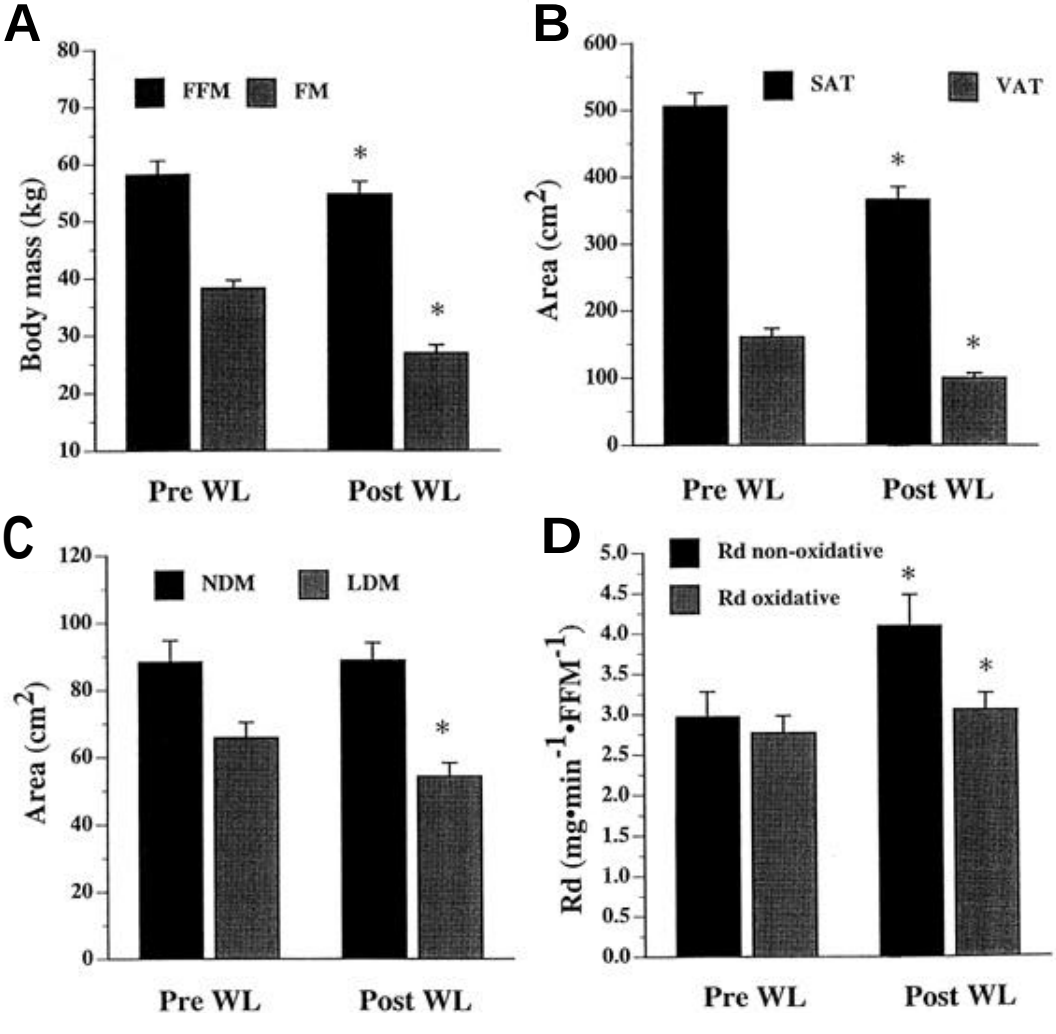

FIG. 2. Effects of WL on FFM and FM (*A*); SAT and VAT (*B*); NDM and LDM (*C*); and nonoxidative and oxidative glucose disposal (R_d) during the clamp (*D*). Values are means ± SE. *$P < 0.01$ vs. pre-WL.

TABLE 1
Changes in whole-body composition in men and women during VLCD compared with lean control subjects

	Women			Men		
	Obese			Obese		
	Before WL	After WL	Lean	Before WL	After WL	Lean
n	17		8	15		7
Weight (kg)	92.5 ± 2.8*	80.3 ± 2.7*†	63.0 ± 4.0	109.0 ± 3.6*	91.4 ± 2.7*†	71.1 ± 4.6
BMI (kg/m^2)	34.0 ± 0.7*	29.5 ± 0.8*†	22.9 ± 1.1	34.5 ± 0.9*	29.0 ± 0.7*†	23.2 ± 1.2
FFM (kg)	49.4 ± 1.9*	47.3 ± 1.7*†	40.7 ± 2.6	70.4 ± 1.9*	65.8 ± 1.7*†	54.6 ± 2.5
FM (kg)	39.1 ± 1.3*	29.6 ± 1.5*†	18.9 ± 2.1	34.6 ± 2.7*	22.3 ± 1.8*†	12.9 ± 3.3
Percent fat	43.0 ± 1.1*	37.1 ± 1.3*†	29.8 ± 2.1	31.7 ± 1.5*	24.2 ± 1.5*†	18.3 ± 1.9

Data are means ± SE. *Significantly different from lean, $P = 0.05$; †significantly different from before WL, $P = 0.05$.

TAT than obese men, while obese men had greater cross-sectional areas of skeletal muscle, as shown in Table 3. After WL, TAT decreased by similar absolute amounts in women and men, representing decreases of 20 ± 3 and 28 ± 2%, respectively. These post-WL values for TAT remained greater than in lean women and men. The loss of thigh fat determined with DEXA was 440 g in women (20% change) and 517 g in men (31% change).

Skeletal muscle in obese subjects, besides being of greater cross-sectional area than in lean subjects, also had an altered composition on CT imaging in terms of lower mean values for its attenuation values (HU), a characteristic previously reported to correlate with insulin sensitivity within cross-sectional comparisons among this cohort (19). Using the histogram of attenuation values of skeletal muscle in lean subjects as normative data, skeletal muscle in the mid-thigh was categorized as being of normal or low density (NDM and LDM). Applying these criteria to the histograms of muscle attenuation in obese subjects (Fig. 3), obese men and women had greater amounts of LDM than lean individuals, both in terms of absolute areas (Table 3) and as percentages of mid-thigh skeletal muscle (46 ± 2.7% in obese compared with 30 ± 2.4% in lean subjects; similar in men and women). However, the cross-sectional area of NDM was similar in obese and lean women, and also in obese and lean men, with a sex-related difference of greater NDM in men. Weight loss had virtually no effect on the cross-sectional areas of NDM in men and effected a slight, though nonsignificant, increase in women. In contrast, there were reductions in LDM after weight loss (Fig. 2), these losses being similar in men and women (Table 3). Across the cohort of obese men and women, the WL-induced changes in mid-thigh cross-sectional skeletal muscle area determined by CT were correlated with changes assessed by DEXA in both systemic FFM ($r = 0.55$, $P < 0.01$) and lower-extremity FFM ($r = 0.56$, $P < 0.01$).

Effects of weight loss on insulin sensitivity. Weight loss significantly improved insulin-stimulated glucose metabolism (Table 4). The improvement in glucose utilization was not statistically different between men and women but, in comparison with the lean cohort, there were some differences between men and women. Among obese men, glucose utilization after WL was not significantly different from that of lean men. Among obese women, glucose utilization after WL also improved significantly, but remained lower than in lean women. The improvement in glucose utilization was accounted for by higher rates of nonoxidative glucose metabolism, with only nominal changes in insulin-stimulated rates of glucose oxidation (Fig. 2).

Within this cohort of normotensive, glucose-tolerant subjects, WL did not significantly change fasting plasma glucose but did reduce fasting levels of plasma insulin, systolic blood pressure, plasma cholesterol, and plasma triglyceride and increased the ratio of HDL to total cholesterol in both men and women (Table 5). Subjects had been asked not to alter patterns of physical activity during the dietary-induced weight loss intervention, and values for $V_{O_{2max}}$ were unchanged compared with pre-WL values. Before WL, plasma leptin levels correlated with percent FM ($r = 0.90$, $P < 0.01$) and were higher in women (Table 5). WL significantly reduced plasma leptin levels in both women and men, although leptin remained higher than in lean subjects. The fall in leptin was proportionate to the loss of adiposity. Change in leptin (induced by WL) correlated significantly with change in total FM ($r = 0.45$, $P < 0.05$), change in visceral fat ($r = 0.36$, $P < 0.05$), change in subcutaneous

TABLE 2
Changes in abdominal adiposity in men and women during VLCD compared with lean control subjects

	Women			Men		
	Obese			Obese		
	Before WL	After WL	Lean	Before WL	After WL	Lean
n	17		8	15		7
Visceral fat (cm^2)	147 ± 14*	103 ± 10*†	51 ± 19	167 ± 19*	89 ± 7†	86 ± 25
Subcutaneous abdominal fat (cm^2)	520 ± 21*	403 ± 22*†	215 ± 33	465 ± 32*	306 ± 24*†	154 ± 42
Total abdominal fat (cm^2)	667 ± 36*	506 ± 31*†	266 ± 39	632 ± 12*	395 ± 17*†	240 ± 30

Data are means ± SE. *Significantly different from lean, $P = 0.05$; †significantly different from before WL, $P = 0.05$.

TABLE 3
Changes in thigh adipose and lean tissue content in men and women during VLCD compared with lean control subjects

	Women			Men		
	Obese			Obese		
	Before WL	After WL	Lean	Before WL	After WL	Lean
n	17		8	15		7
Thigh lean tissue (cm^2)	266 ± 10*	252 ± 12*†	222 ± 15	370 ± 10*	340 ± 8†	311 ± 16
Thigh fat (cm^2)	418 ± 18*	328 ± 20†	261 ± 28	310 ± 24*	224 ± 14*†	108 ± 30
Muscle attenuation (HU)	34.7 ± 1.0*	37.2 ± 1.1†	38.6 ± 1.5	35.9 ± 1.3	37.6 ± 1.3	39.7 ± 1.9
LDM (cm^2)	122 ± 12*	94 ± 8†	68 ± 4	164 ± 14*	134 ± 14	93 ± 17
NDM (cm^2)	142 ± 12	158 ± 14	154 ± 12	204 ± 14	206 ± 14	217 ± 38

Data are means ± SE. *Significantly different from lean, $P \leq 0.05$; †significantly different from before WL, $P \leq 0.05$.

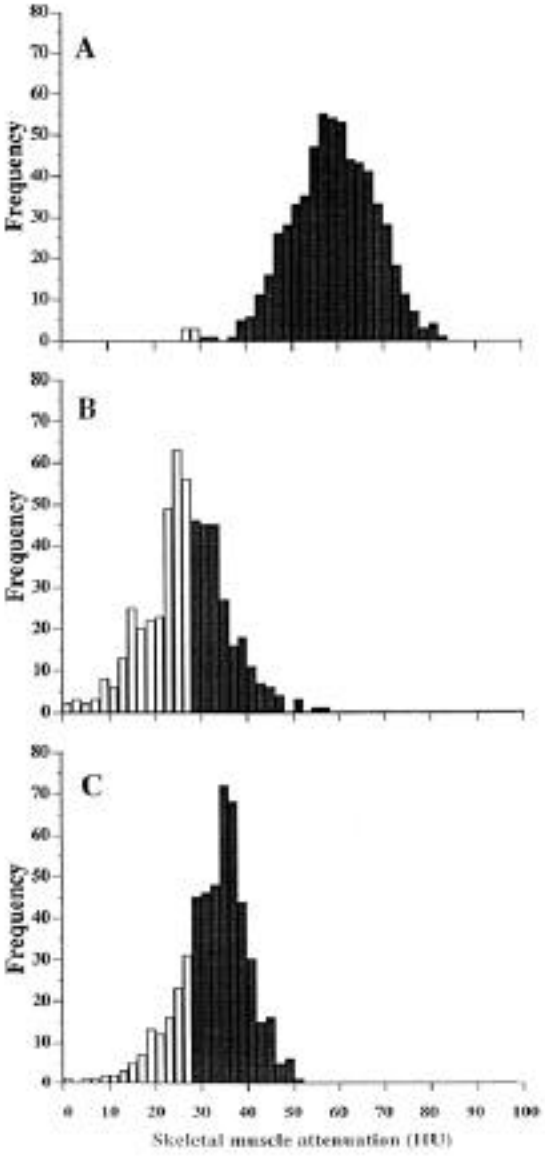

FIG. 3. Representative histograms of muscle attenuation characteristics determined by CT in lean subjects (*A*) and in obese subjects before (*B*) and after (*C*) WL. Open bars demonstrate the frequency of pixels from 0–29 HU (LDM); filled bars demonstrate the frequency of pixels from 30–100 HU (NDM). In the lean individual, very few pixels are in the LDM range, and after WL, a lower amount of muscle is represented by LDM.

abdominal fat (r = 0.39, P < 0.05), and the amount of decline in fasting insulin (r = 0.40, P < 0.05).

Relation of weight loss to improvement in insulin sensitivity. After weight loss, significant correlations between R_d and adiposity persisted, as shown in Fig. 4. When values for post-WL R_d are plotted against FM or VAT, the relationship essentially falls along the same regression lines determined by plotting respective values from lean and pre-WL obese subjects. However, the amount of decrease in adiposity did not correlate significantly with the degree of improvement in R_d, except in regard to VAT. Using improvement in R_d as the dependent variable, simple correlations were examined with respect to change in weight (r = 0.14); change in FM (r = 0.04); and changes in VAT (r = 0.30), SAT (r = 0.03) and TAT (r = 0.02). Among those, only the correlation between change in VAT and change in R_d approached statistical significance (P = 0.11). Similar results were obtained if percent change in adiposity (regional and total) was examined; in this analysis as well, only the percent change in VAT was significantly correlated with the improvement in R_d (r = 0.37, P < 0.05). Similar regression analyses were performed using the improvement in nonoxidative glucose disposal, since this was the component of insulin-stimulated glucose metabolism that showed the greatest improvement after WL. Again, the reduction in VAT held the strongest single correlation with improvement in nonoxidative glucose disposal (r = 0.36, P < 0.05); other fat depot changes did not relate significantly. Alhough men lost more VAT and exhibited greater improvements in R_d than women, the associations between the loss of VAT and degree improvement in R_d were not influenced by sex.

Before weight loss, the mid-thigh muscle attenuation and the percent of skeletal muscle area as LDM were associated with R_d (r = 0.42, P < 0.01, and r = 0.40, P < 0.01, respectively). As noted above, muscle attenuation increased and the percentage of thigh muscle attributed to LDM decreased after weight loss; however, including post-WL values of these characteristics of muscle composition in regression against R_d tended to weaken the correlations (muscle attenuation vs. R_d, r = 0.26, P = 0.02; LDM vs. R_d, r = –0.29, P = 0.01). Furthermore, neither the amount of increase in muscle attenuation nor the amount of reduction in LDM after WL had a significant correlation with the corresponding amount of improvement in R_d.

DISCUSSION

A strong association between obesity and insulin resistance has been recognized for many years (25), and it is now gen-

TABLE 4
Changes in insulin-stimulated glucose utilization in men and women during VLCD compared with lean control subjects

	Women			Men		
	Obese			Obese		
	Before WL	After WL	Lean	Before WL	After WL	Lean
n	17		8	15		7
R_d (mg · FFM^{-1} · min^{-1})	6.2 ± 0.5*	7.3 ± 0.6*†	9.9 ± 0.9	5.5 ± 0.6*	7.4 ± 1.1†	8.8 ± 0.5
Nonoxidative	3.1 ± 0.5*	3.9 ± 0.5†	5.5 ± 0.8	2.9 ± 0.4*	4.7 ± 0.9†	5.3 ± 0.6
Oxidative	3.1 ± 0.3*	3.4 ± 0.3*	4.4 ± 0.4	2.6 ± 0.3	2.7 ± 0.2	3.6 ± 0.3

Data are means ± SE. *Significantly different from lean, $P \leq 0.05$; †significantly different from before WL, $P \leq 0.05$.

erally accepted that regional patterns of fat deposition, especially abdominal adiposity, relate strongly to insulin resistance (6,8,19,26–28). In support of these associations between body composition and metabolism, there is a growing body of data that has begun to more clearly delineate mechanisms linking adiposity and insulin resistance (29–39). Thus, there is a growing biological basis for understanding the mechanisms by which weight loss alleviates insulin resistance. In the current obese cohort, before a weight loss intervention there were fairly strong associations between regional adiposity and insulin resistance, as we previously reported (19). In that report (19), a relatively strong association between the amount of abdominal adiposity and the severity of insulin resistance was noted. Of interest, we observed that subcutaneous abdominal adipose tissue held as strong a correlation to insulin resistance as did visceral adipose tissue, and this observation is in general accord with the recent findings of Abate et al. (6,26). Such findings call into question whether visceral adiposity, a depot that is substantially smaller in volume than subcutaneous abdominal fat, is of singular importance in the pathophysiology of obesity-related insulin resistance. A more rigorous manner to examine this issue is by a weight loss intervention. In the current study, we have used this approach to examine the relationship between abdominal fat distribution and insulin resistance. Fujioka et al. (15) found that improvements in glucose tolerance after dietary-induced weight loss were significantly related to decreases in visceral adipose tissue, even after adjusting for overall weight loss. To our knowledge, however, the present study is the first to examine the relationship between WL-induced changes in regional adiposity and changes in insulin sensitivity, measuring this parameter using insulin infusion studies.

In the current study, we found substantial changes in total and regional adiposity after weight loss. The largest change was in visceral adipose tissue, which decreased by ~40%, compared with an average decrease in overall fat mass of ~30% and of subcutaneous abdominal adipose tissue of ~30%. Zamboni et al. (14) found that VAT was decreased by 44% in premenopausal women during weight loss compared with a 24% decrease in SAT, and data by van der Kooy et al. (40) examining both men and women are in substantial agreement. Thus, the findings of the current study further support the concept that VAT is more effectively depleted by weight loss than is SAT. The percentage of decrease in our study is indeed very close to those reported earlier (11,14,40).

In obesity, the accretion of weight entails not only increased fat mass but also increased fat-free mass, and weight loss also entails loss of fat-free mass as well as adipose tissue. One of the objectives of the current study was to examine the effects of weight loss on skeletal muscle composition in obesity. Based on compartmental analysis of body composition using DEXA, women and men lost modest amounts of fat-free mass during weight loss, to a somewhat greater extent in men. Using CT to more specifically examine skeletal muscle, with imaging at the level of the mid-thigh, a similar pattern of modest decrease was observed after

TABLE 5
Selected clinical characteristics before and after weight loss in men and women compared with lean control subjects

	Women			Men		
	Obese			Obese		
	Before WL	After WL	Lean	Before WL	After WL	Lean
n	17		8	15		7
$V_{O_{2max}}$ (mg · FFM^{-1} · min^{-1})	36.6 ± 0.9	36.4 ± 1.0	42.3 ± 1.5	42.1 ± 1.1	43.9 ± 1.0	44.1 ± 1.2
Glucose (mmol/l)	4.7 ± 0.08	4.6 ± 0.04	4.6 ± 0.16	4.8 ± 0.12	4.6 ± 0.09	4.6 ± 0.1
Insulin (mU/ml)	17.7 ± 2.9*	9.9 ± 1.1†	7.4 ± 1.6	15.5 ± 2.4*	7.2 ± 0.7†	5.8 ± 0.8
Cholesterol						
Total (mg/dl)	193 ± 9	178 ± 10†	178 ± 18	202 ± 12	170 ± 13†	187 ± 13
HDL (mg/dl)	46 ± 2	46 ± 2	49 ± 5	47 ± 2	43 ± 2*†	52 ± 5
HDL:total	0.24 ± 0.02	0.27 ± 0.01†	0.28 ± 0.04	0.24 ± 0.02	0.27 ± 0.02†	0.29 ± 0.04
Triglyceride (mg/dl)	147 ± 19*	105 ± 10†	83 ± 12	162 ± 23	116 ± 20†	147 ± 35
Leptin (ng/ml)	38.5 ± 2.3*	21.1 ± 1.8*†	14.7 ± 2.6	16.3 ± 2.5*	5.5 ± 2.0†	3.1 ± 2.8

Data are means ± SE. *Significantly different from lean, $P \leq 0.05$; †significantly different from before WL, $P \leq 0.05$.

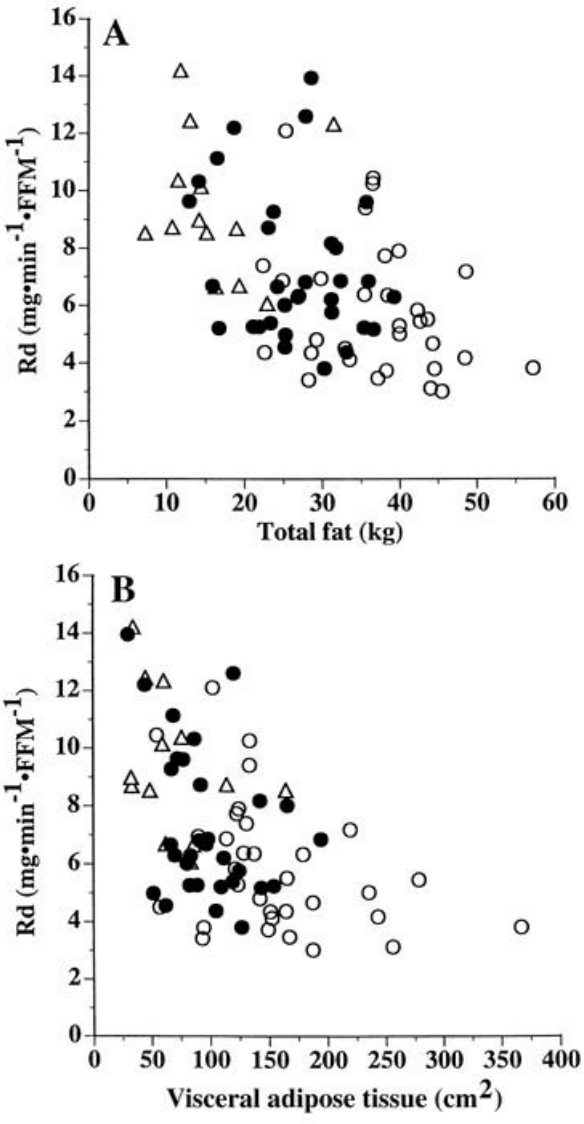

FIG 4. Associations of total body fat (A) and visceral adipose tissue (B) with insulin sensitivity (R_d) before and after WL. △, lean control subjects; ○, obese subjects before WL; ●, obese subjects after WL. Total body fat versus R_d was r = –0.56, P < 0.01 before WL and r = –0.36, P = 0.02 after WL. Visceral adipose tissue area versus R_d was r = –0.54, P < 0.01 before WL and r = –0.42, P < 0.01 after WL.

weight loss. Moreover, weight loss altered muscle composition. As ascertained by the distribution of CT attenuation values within skeletal muscle, after weight loss there was a substantial decrease in skeletal muscle comprising low attenuation values, yet virtually no change in the amount of skeletal muscle within the normal range of attenuation values, the normal range being defined on the basis of prior studies in lean individuals (41). As previously noted, obese subjects before weight loss had an increased amount (and proportion) of skeletal muscle comprising lower attenuation values (19,41), findings that reflect increased lipid content within muscle fibers (18). The current findings indicate that a moderate amount of dietary-induced weight loss selectively depletes low-density skeletal muscle in obese individuals.

Among the 32 subjects who completed the weight loss intervention, insulin sensitivity improved considerably, by ~25%. This improvement of insulin-stimulated glucose metabolism was mostly due to enhancement of nonoxidative glucose disposal, a finding consistent with prior studies (2–5,15,42,43). After weight loss, men had insulin sensitivity that did not differ from that of lean men, despite the fact that a lean BMI was not attained. Women after weight loss remained more insulin resistant than lean control subjects, but there was not a statistically significant sex-related effect discerned in the improvement of insulin sensitivity after weight loss. In regression analysis of our data, plotting VAT against insulin sensitivity, post-WL values essentially fall along the same regression determined by data of lean and obese (pre-WL) individuals, as shown in Fig. 4. This result suggests that the relationship between VAT and insulin resistance persists after weight loss; further, consistent with this concept, the percent change in VAT correlated significantly with the change in insulin sensitivity. However, changes in insulin sensitivity did not significantly relate to the decreases measured in other regional depots of adiposity, including that of subcutaneous abdominal adipose tissue and thigh muscle composition. Nor was there a significant correlation between the decrease (absolute or relative) of fat mass and the change in insulin sensitivity. The most straightforward explanation for these results is that visceral adipose tissue has a stronger relationship with obesity-related insulin resistance than systemic adiposity or subcutaneous abdominal adiposity.

The concept that visceral adiposity is of particular importance in obesity-related insulin resistance is bolstered by observation that in men, weight loss improved insulin sensitivity and reduced visceral adiposity to levels similar to lean men, yet no other parameter of adiposity in obese men was reduced to the levels found among lean control subjects. From a clinical perspective, these observations point to the importance of diminished visceral obesity and, from a conceptual viewpoint, weaken the notion developed from cross-sectional studies, including a recent one from our laboratories (19), that subcutaneous abdominal fat is of equivalent clinical significance in relation to insulin resistance. Nevertheless, the change in VAT only accounted for ~15% of the improvement in insulin sensitivity, so undoubtedly factors other than change in the size of adipose tissue depots mediate WL-induced improvement. Inability to predict changes in insulin sensitivity from amount of weight was recently noted in non-diabetic obese subjects by Wing (44). Although change in weight at 1 year did relate to improvement in insulin sensitivity in subjects with type 2 diabetes (44), those results were confounded by weight regain. Methodologic limitations in detecting accurate changes in body composition are not likely to be an explanation for the lack of association between reduced adiposity and improvements in insulin sensitivity. In the current study, changes in adiposity, regional and systemic, correlated significantly with changes in leptin, a relationship one would logically expect to exist; in addition, our laboratory has previously demonstrated a high degree of reproducibility for measurement by computed tomography of visceral and subcutaneous abdominal fat (45).

Another perspective is that the amount of weight loss attained in the current study exceeded a threshold value needed for improvement of insulin resistance. Current therapeutic recommendations are to achieve a 5–10% weight loss, rather than striving to attain a lean weight. A number of studies indicate that a 5–10% loss of weight can achieve clin-

ically significant improvement in cardiovascular risk factors such as hypertension, glucose intolerance, and dyslipidemia (46,47). In the current study, the average weight loss was ~15% of initial weight, none of the subjects lost <5%, and only 4 of the 32 subjects lost <10% of initial weight. Perhaps the key is to achieve loss of a crucial amount of visceral adiposity and, since this depot is reduced to a greater extent than others, modest weight loss is sufficient to achieve this goal.

In summary, after a substantial weight loss attained by calorie restriction and without collateral changes in aerobic fitness, there are significant increases in insulin sensitivity and significant decreases in total and regional adiposity. Among these various changes in body composition, including changes in the composition of muscle itself, the percent change in visceral adiposity related most clearly to improved insulin sensitivity. These observations from weight loss intervention reemphasize both the conceptual and clinical importance of visceral adiposity in relation to obesity-induced insulin resistance.

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