

# Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus<sup>1-3</sup>

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## ABSTRACT

**Background:** Adipose tissue (AT) content of the thigh is generally not considered to be associated with insulin resistance (IR), but it is unclear whether the distribution of AT in the thigh is a determinant of IR.

**Objective:** We investigated whether subcompartments of AT within the thigh are determinants of IR.

**Design:** Midthigh AT, muscle composition, and insulin sensitivity were compared in 11 obese patients with type 2 diabetes mellitus (DM); 40 obese, glucose-tolerant (GT) and 15 lean, GT volunteers; and 38 obese subjects who completed a weight-loss program. Midthigh AT area measured with computed tomography was partitioned into 3 components: subcutaneous AT (SCAT), AT beneath the fascia (SFAT), and AT infiltrating muscle groups (IMAT). Muscle attenuation characteristics were determined.

**Results:** Obese DM and obese GT subjects had lower insulin sensitivity than lean GT subjects. SCAT was greater in obesity, yet did not correlate with insulin sensitivity. SFAT was  $\approx 8\%$  of total thigh AT and correlated with insulin sensitivity. IMAT was highest in obese DM, and although it accounted for only  $\approx 3\%$  of thigh AT, it was a strong correlate of insulin sensitivity. Mean attenuation was highest in lean subjects and was associated with higher insulin sensitivity. Weight loss reduced the amount of thigh AT, the proportion of thigh IMAT, and the amount of low-density thigh muscle.

**Conclusions:** SFAT and IMAT are markers of IR in obesity and DM although they are much smaller than SCAT, which does not predict IR. Muscle composition reflecting increased fat content is also associated with IR. *Am J Clin Nutr* 2000;71:885-92.

**KEY WORDS** Insulin resistance, skeletal muscle, adipose tissue, weight loss, thigh adipose tissue, thigh muscle, obesity, insulin sensitivity, type 2 diabetes mellitus, non-insulin-dependent diabetes mellitus, adult-onset diabetes mellitus, computed tomography, glucose tolerance, body composition, regional fat distribution

## INTRODUCTION

Regional fat distribution, particularly a high proportion of upper-body or abdominal fat, is recognized to be an important component in the insulin resistance of obesity and type 2 diabetes mellitus (DM) (1-7). Conversely, there is little direct evidence linking thigh adipose tissue (AT) content with insulin resistance.

Carey et al (8) showed that thigh fat content as measured by dual-energy X-ray absorptiometry (DXA) was not associated with insulin resistance in overweight women. In addition, Sparrow et al (7) showed that the total cross-sectional area of thigh AT determined with computed tomography (CT) was not associated with glucose tolerance (7). However, not all fat accumulation within the thigh is subcutaneous; AT can also accumulate beneath the fascia lata and within muscle itself (9-11). This suggests that fat distribution within the thigh, particularly that located adjacent to or within skeletal muscle, may be a previously unrecognized component of regional fat deposition that is associated with insulin resistance. Several studies showed that increased skeletal muscle lipid content is associated with the severity of insulin resistance (12-14). We observed a strong relation between increased muscle fat content (reduced muscle attenuation on CT) and insulin resistance independent of total and visceral adiposity (14). Whether there is an association between the AT interspersed within muscle or beneath the fascia and insulin resistance has not been examined previously. Consequently, we sought to test the hypothesis that compartmentalization of thigh AT, ie, AT deposition between and around thigh skeletal muscle, is an important marker of insulin resistance in obesity and DM.

Weight-loss interventions are commonly prescribed to obese individuals to improve insulin sensitivity and reduce risk factors associated with metabolic disease. Although many investigations have focused on the effects of weight loss on abdominal AT distribution (15-18), little is known about the effects of weight loss on thigh AT distribution or changes in skeletal muscle fat content. In a recent study (19), we showed that in a group of glucose-tolerant (GT) obese subjects, an average weight loss of 15 kg induced by energy restriction increased muscle attenuation and decreased total thigh AT content. However, the various AT

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compartments within the thigh that can be identified with CT were not determined in that study. Therefore, the current study examined the effects of weight loss on thigh AT distribution and skeletal muscle composition in obese GT subjects and obese individuals with type 2 DM.

## SUBJECTS AND METHODS

### Subjects

The volunteers were 15 lean GT individuals (8 women and 7 men), 40 obese GT individuals (21 women and 19 men), and 11 obese patients with type 2 DM (6 women and 5 men). The clinical characteristics of the subjects are shown in **Table 1**. Individuals with hyperlipidemia (concentrations of plasma triacylglycerol >3.95 mmol/L or total cholesterol >7.76 mmol/L), coronary heart disease, vascular disease, or hypertension were excluded, as were individuals taking antihypertensive agents. Subjects with DM discontinued their oral antidiabetic medications for 2 wk before the study, and none had received insulin. None of the subjects engaged in any regular exercise. The protocol was approved by the University of Pittsburgh Institutional Review Board, and all subjects gave their written, informed consent.

### Weight loss induced by energy restriction

A subgroup of obese individuals with a body mass index (BMI; in kg/m<sup>2</sup>) ≥30 was asked to participate in a weight-loss program designed to achieve a 15-kg weight reduction via energy restriction. The energy restriction lasted for 12 wk; body weight was then carefully stabilized for an additional 4 wk, after which post-weight-loss assessments were performed at week 17. The program was described previously (19); subjects consumed a very-low-energy diet (3.35 MJ/d) consisting of liquid formula (Novartis, Minneapolis) and lean meat, fish, and poultry. Of the volunteers, 28 obese GT individuals (15 women and 13 men) and 10 obese individuals with DM (5 men and 5 women) completed the program and 5 subjects dropped out.

### Body composition

#### Dual-energy X-ray absorptiometry

Whole-body fat mass and fat-free mass were assessed with DXA (model DPX-L; Lunar Corp, Madison, WI) by using proprietary software (version 1.3Z; Lunar Corp). Fat mass of the midthigh was determined with DXA within a manually drawn rectangular region of interest with a width spanning the thighs. The height of the region of interest was calculated for each individual as exactly half the distance between the top of the iliac crest and the patella, centered at this midpoint.

#### Computed tomography

To characterize the amount and location of AT within the thigh, cross-sectional CT imaging (model 9800 CT scanner; General Electric, Milwaukee) was performed. With the subject in a supine position, we obtained a 10-mm cross-sectional scan of both legs at the midpoint between the anterior superior iliac crest and the patella. Scans were obtained by using a 512 × 512 matrix and a 48-cm field of view, thereby attaining a pixel resolution of 0.94 mm. AT area was measured in the right thigh in the range of -190 to -30 Hounsfield units (HU) (4) by using proprietary software (GE

**TABLE 1**

Clinical characteristics of lean glucose-tolerant (GT) and obese GT subjects and obese subjects with type 2 diabetes mellitus (DM)<sup>1</sup>

	Lean GT (n = 15)	Obese GT (n = 40)	Obese DM (n = 11)
Age (y)	34.1 ± 1.6	37.2 ± 1.0	45.5 ± 2.1 <sup>2</sup>
Weight (kg)	67.8 ± 2.3	99.0 ± 2.6 <sup>2</sup>	102.7 ± 5.6 <sup>2</sup>
BMI (kg/m <sup>2</sup> )	23.4 ± 0.6	33.6 ± 0.6 <sup>2</sup>	36.0 ± 1.1 <sup>2</sup>
Total body fat mass (kg)	12.9 ± 0.8	33.8 ± 1.8 <sup>2</sup>	39.4 ± 0.7 <sup>2,3</sup>
Insulin sensitivity (mg · min <sup>-1</sup> · kg FFM <sup>-1</sup> )	9.4 ± 0.7	5.9 ± 0.3 <sup>2</sup>	3.6 ± 0.3 <sup>2,3</sup>
Serum insulin (pmol/L)	35 ± 14	89 ± 11 <sup>2</sup>	139 ± 26 <sup>2,3</sup>
Plasma glucose (mmol/L)	4.6 ± 0.3	4.8 ± 0.5	10.8 ± 1.1 <sup>2,3</sup>

<sup>1</sup> $\bar{x} \pm SE$ ; FFM, fat-free mass. Differences among groups were determined with ANOVA followed by a Tukey's post hoc pairwise analysis for specific group differences.

<sup>2</sup>Significantly different from lean GT subjects,  $P \leq 0.01$  (ANOVA).

<sup>3</sup>Significantly different from obese GT subjects,  $P \leq 0.01$  (ANOVA).

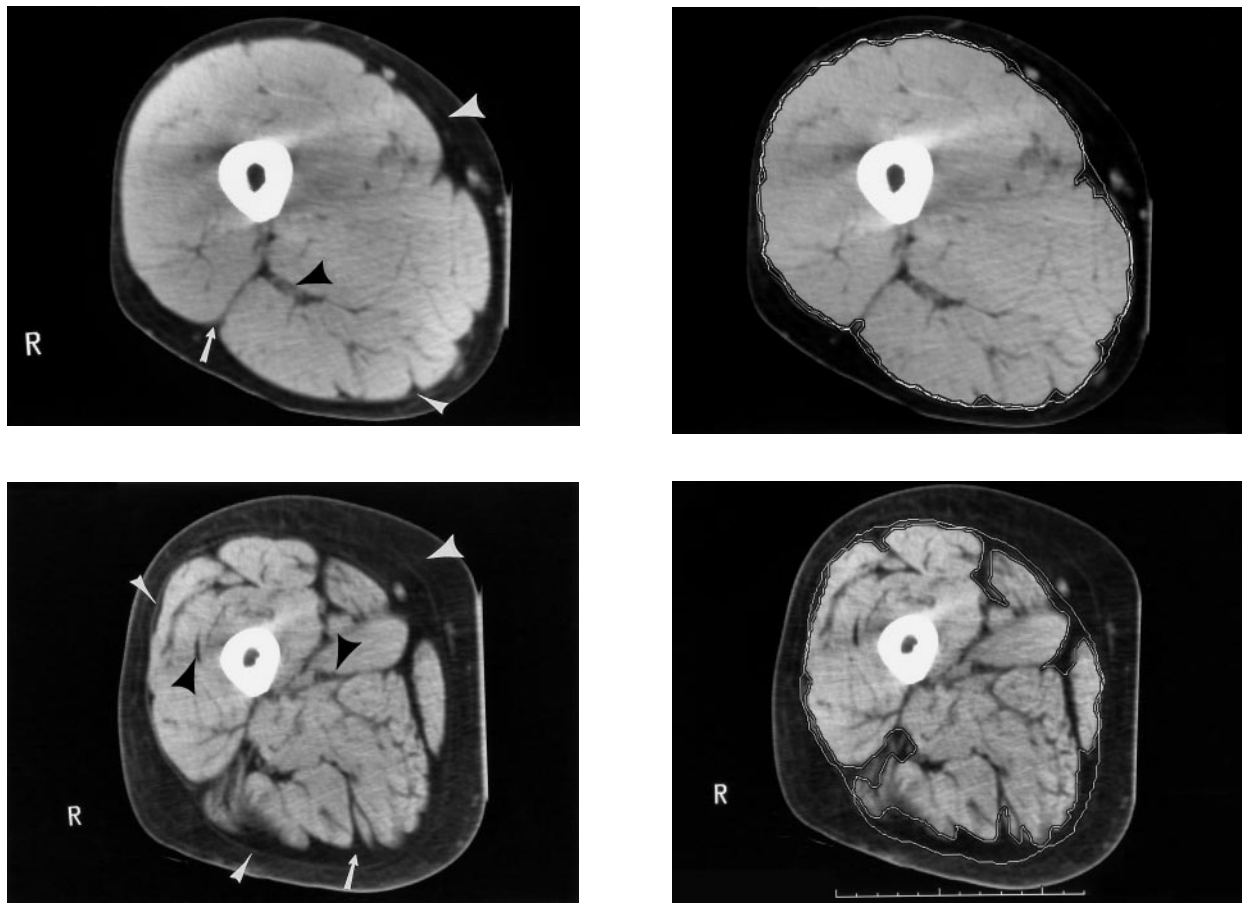
Medical Systems, Milwaukee). Three compartments of thigh AT cross-sectional area were separated by manual tracings (**Figure 1**). One tracing was drawn around the outermost edge of the thigh skeletal muscle to distinguish intermuscular adipose tissue (IMAT). Next, the window level was adjusted to visualize the fascia lata surrounding skeletal muscle and a manual tracing was drawn to differentiate subcutaneous adipose tissue (SCAT) from adipose tissue between the fascia and muscle (subfascial AT; SFAT). Bone marrow AT was excluded.

Skeletal muscle attenuation was determined by measuring the mean attenuation value from all pixels within the range of 0 to 100 HU. As an additional means of characterizing muscle composition, the distribution of attenuation values was described as representing 2 components on the basis of muscle density. One component, normal-density muscle (NDM), was defined as muscle pixels with attenuation values within 2 SDs of the mean attenuation value observed in lean, normal muscle (31–100 HU). The second component, low-density muscle (LDM), was defined as muscle with below-normal attenuation values (0–30 HU). These methods have been described previously (20).

### Insulin sensitivity

We determined insulin sensitivity by using the hyperinsulinemic euglycemic clamp method as described previously (14). Subjects were instructed to consume a weight-maintaining diet containing ≥200 g carbohydrate for ≥3 d before measurements of insulin sensitivity and to avoid strenuous activity for 2 d preceding these 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 providing 42 kJ/kg with 50% of energy from carbohydrate, 30% from fat, and 20% from protein. After this meal, they fasted until completion of the glucose and insulin infusions. At ≈0700, a catheter was placed in a forearm vein to start a primed, continuous infusion of [3-<sup>3</sup>H]glucose (New England Nuclear, Boston) 100 min before beginning the euglycemic insulin infusion; the insulin dose was 40 mU · m<sup>-2</sup> · min<sup>-1</sup>, where m<sup>2</sup> represents body surface area (Humulin; Eli Lilly, Indianapolis). Tracer was given to allow determination of glucose utilization during the final 30 min of the 3-h insulin infusion. An additional catheter was inserted into a





**FIGURE 1.** Representative midthigh computed tomography images, in a lean volunteer (upper panels) and an obese volunteer (lower panels), illustrating compartments of subcutaneous (large white arrowhead), subfascial (white arrow), and intermuscular (large black arrowhead) adipose tissue. The fascia lata (small white arrowhead) was defined in all images by manual tracing (right-hand panels).

radial artery for blood sampling. Baseline postabsorptive arterial samples were collected for determination of serum insulin concentration. Euglycemia was maintained by using an adjustable infusion of 20% dextrose to which [ $3\text{-}^3\text{H}$ ]glucose was added to maintain stable plasma glucose specific activity (21). Plasma glucose concentration was determined at 5-min intervals during the clamp procedure. Blood samples for measurement of [ $3\text{-}^3\text{H}$ ]glucose specific activity were collected every 10 min during the final 40 min of insulin infusion.

#### Glucose and insulin analyses

Plasma glucose concentrations were measured by using an automated glucose oxidase reaction (YSI 2300 glucose analyzer; Yellow Springs Instrument Co, 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 concentrations were determined by using commercially available radioimmunoassay kits (Pharmacia, Uppsala, Sweden).

#### Calculations

The rates of plasma glucose appearance and utilization were calculated by using the Steele equations (22) as modified for variable-rate glucose infusions that contain isotope (21). The rate

of insulin-stimulated glucose utilization was used as the measure of insulin sensitivity.

#### Statistical analysis

The data are presented as means ( $\pm$ SEM) unless otherwise indicated. Regression analysis was used to determine the associations between insulin sensitivity and thigh AT content, muscle attenuation, and areas of NDM and LDM. For certain variables, the lean GT, obese GT, and obese DM groups were compared with analysis of variance, and pairwise group differences were assessed with Tukey's post hoc analysis. Weight changes were compared by using paired *t* tests. All statistical analyses were performed by using JMP version 3.1.6 for the Macintosh (SAS Inc, Cary, NC).

## RESULTS

#### Total fat mass and insulin sensitivity

Obese subjects had 2- to 3-fold greater fat mass, as determined by DXA, than did lean subjects ( $P < 0.01$ ). Obese DM subjects had significantly greater fat mass than obese GT subjects (Table 1). Obese subjects were hyperinsulinemic and were more insulin-resistant than lean subjects ( $P < 0.01$ ). Obese DM subjects were further distinguished by fasting hyperglycemia,

**TABLE 2**

Midthigh adipose tissue distribution and skeletal muscle composition in lean glucose-tolerant (GT) and obese GT subjects and in obese subjects with type 2 diabetes mellitus<sup>1</sup>

	Lean GT (n = 15)	Obese GT (n = 40)	Obese DM (n = 11)
SCAT (cm <sup>2</sup> )	86 ± 13	144 ± 8 <sup>2</sup>	134 ± 15 <sup>2</sup>
SFAT (cm <sup>2</sup> )	6 ± 2	13 ± 1 <sup>2</sup>	15 ± 3 <sup>2</sup>
IMAT (cm <sup>2</sup> )	1 ± 1	5 ± 1 <sup>2</sup>	9 ± 2 <sup>2</sup>
Muscle attenuation (HU)	39.2 ± 1.7	35.9 ± 1.9 <sup>2</sup>	33.9 ± 1.2 <sup>2</sup>
NDM (cm <sup>2</sup> )	181 ± 6	180 ± 12	175 ± 15
LDM (cm <sup>2</sup> )	81 ± 13	137 ± 8 <sup>2</sup>	142 ± 15 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm SE$ . SCAT, subcutaneous adipose tissue; SFAT, subfascial adipose tissue; IMAT, intermuscular adipose tissue; NDM, normal-density muscle; LDM, low-density muscle; HU, Hounsfield unit. Values are pre-weight loss for obese subjects.

<sup>2</sup>Significantly different from lean GT subjects,  $P \leq 0.01$  (ANOVA with Tukey's post hoc comparisons).

greater insulin resistance, and higher fasting plasma insulin concentrations than in obese GT volunteers. Men and women had similar amounts of fat mass ( $30.1 \pm 2.1$  and  $34.4 \pm 2.1$  kg, respectively), and insulin sensitivity did not differ significantly between men and women ( $5.74 \pm 0.50$  and  $6.79 \pm 0.48$  mg · min<sup>-1</sup> · kg fat-free mass<sup>-1</sup>, respectively).

### Thigh adipose tissue

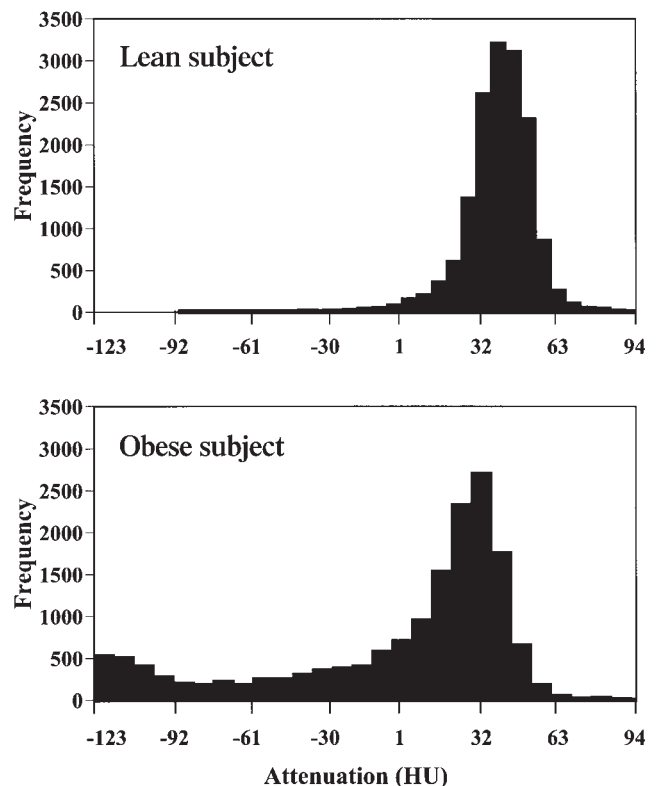
Total thigh AT, defined by HU in the range of -190 to -30 on CT, was divided into 3 compartments and measured as areas of thigh SCAT, SFAT, and IMAT (Figure 1). Both the obese GT and obese DM groups had greater ( $P < 0.01$ ) cross-sectional areas of SCAT, SFAT, and IMAT than the lean GT group (Table 2). SCAT represented the majority of total thigh AT area (90%), whereas SFAT and IMAT each represented a much smaller proportion of total thigh AT area (8% and 2%, respectively). Representative histograms (Figure 2) of CT attenuation values illustrate that very little AT was visible as IMAT in lean individuals, whereas a typical obese volunteer had a substantial amount of IMAT. Simple linear regression analysis revealed that SCAT, SFAT, and IMAT were all significantly correlated with fat mass (Table 3). Men and women had similar amounts of SFAT and IMAT, but women had more SCAT than men ( $156 \pm 8$  compared with  $101 \pm 9$  cm<sup>2</sup>, respectively).

Despite the relatively small area accounted for by IMAT, there was a strong negative association between IMAT and insulin sensitivity (Figure 3). The proportion of total thigh AT area represented by IMAT was also associated with insulin sensitivity ( $r = -0.45$ ,  $P < 0.01$ ). A similar, albeit slightly weaker association was found between SFAT and insulin sensitivity ( $r = -0.36$ ,  $P < 0.01$ ). These results were also consistent with respect to sex; insulin sensitivity was negatively associated with IMAT ( $r = -0.60$  and  $-0.33$ ,  $P < 0.05$ ) and with SFAT ( $r = -0.38$  and  $-0.36$ ,  $P < 0.05$ ) in men and women, respectively. However, these associations were not significant after adjusting for total body fat. No association was observed between insulin resistance and the relatively large SCAT depot. Total cross-sectional thigh AT by CT was strongly associated with midthigh fat content determined with DXA ( $r = 0.93$ ,  $P < 0.01$ ). However, neither of these measures of total thigh adiposity correlated with insulin sensitivity ( $r = -0.17$  and  $-0.23$  for CT and DXA, respectively).

Adjustment for age did not affect the correlations between thigh AT content and insulin resistance.

### Skeletal muscle composition

The attenuation characteristics of midthigh skeletal muscle were also determined with CT. Lower attenuation values as measured with CT indicate greater fat content within muscle because lipid is characterized by negative attenuation values on CT. Muscle attenuation was positively associated with insulin sensitivity ( $r = 0.41$ ,  $P < 0.01$ ) and these associations were significant in men ( $r = 0.61$ ,  $P < 0.01$ ) and women ( $r = 0.37$ ,  $P < 0.05$ ). Muscle attenuation was negatively associated with SCAT, SFAT, and IMAT (Table 3). Muscle composition was further characterized as muscle area with attenuation values within the normal range (NDM) or muscle area with attenuation values  $> 2$  SD below the normal range, ie, LDM. Cross-sectional areas of NDM were similar in the lean and obese groups (Table 2), but areas of LDM were greater in the obese GT and obese DM groups ( $P < 0.01$ ). As shown in the histograms in Figure 2, the thigh of a typical obese volunteer registered more pixels in the range representing LDM than the thigh of a typical lean volunteer. The area of LDM in obese subjects was negatively associated with insulin sensitivity ( $r = -0.50$ ,  $P < 0.01$ ); this association was similar for men ( $r = -0.60$ ,  $P < 0.01$ ) and women ( $r = -0.54$ ,  $P < 0.01$ ) and was not influenced by age. Furthermore, multivariate



**FIGURE 2.** Representative histograms of the distribution and frequency of pixels across the range of attenuation values for adipose tissue (AT) (-190 to -30 Hounsfield units, HU) and skeletal muscle (0-100 HU) in a lean subject and an obese subject. Attenuation values were measured in a region of interest that excluded subcutaneous AT and subfascial AT, thus including only intermuscular AT and muscle. Neither histogram registered pixel values  $< -123$  HU or  $> 94$  HU.

**TABLE 3**

Correlations between insulin sensitivity, total fat mass, muscle attenuation, and thigh adipose tissue (AT) areas

	Insulin sensitivity	Fat mass	Muscle attenuation
	$\text{mg} \cdot \text{min}^{-1} \cdot \text{kg FFM}^{-1}$	kg	HU
SCAT ( $\text{cm}^2$ )	-0.12	$0.73^2$	$-0.35^2$
SFAT ( $\text{cm}^2$ )	$-0.36^2$	$0.68^2$	$-0.42^2$
IMAT ( $\text{cm}^2$ )	$-0.45^2$	$0.62^2$	$-0.58^2$
Total thigh AT ( $\text{cm}^2$ )	-0.17	$0.86^2$	$-0.44^2$
Total thigh AT by DXA (g)	-0.23	$0.87^2$	$-0.33^2$
Muscle attenuation (HU)	$0.41^2$	$-0.47^2$	

<sup>1</sup>HU, Hounsfield unit; FFM, fat-free mass; SCAT, subcutaneous AT; SFAT, subfascial AT; IMAT, intermuscular AT; DXA, dual-energy X-ray absorptiometry.

<sup>2</sup>Simple linear regression ( $n = 68$ ),  $P \leq 0.01$ .

regression analysis revealed that the area of LDM and total body fat independently contributed to the variance in insulin sensitivity; LDM accounted for 30% of the variance and total body fat accounted for an additional 12% of the variance. The area of NDM was not significantly associated with insulin sensitivity.

#### Effects of weight loss

The 16-wk weight-loss program was effective in reducing body weight and total fat mass; the average losses were 15 kg of body weight and 11.2 kg of fat mass ( $P < 0.01$  for both). BMI decreased from  $34.3 \pm 0.6$  to  $29.3 \pm 0.6$  in obese GT subjects and from  $36.2 \pm 1.2$  to  $31.7 \pm 1.2$  in obese DM subjects. Fasting insulin concentrations decreased to  $52 \pm 4$  and  $96 \pm 17$  pmol/L in obese GT and obese DM subjects, respectively. Weight loss increased insulin sensitivity by 27% in obese GT subjects and by 36% in obese DM subjects (to  $7.5 \pm 0.8$  and  $4.9 \pm 0.6$   $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg fat-free mass}^{-1}$ , respectively;  $P < 0.01$  for both). Fasting hyperglycemia was nearly normalized ( $6.3 \pm 0.6$  mmol/L) in obese DM subjects after weight loss ( $P < 0.01$ ).

With weight loss, the SCAT and SFAT compartments were reduced to a similar extent (22% and 18%, respectively;  $P < 0.01$  for both) (Table 4). However, the proportion of IMAT lost (36%)

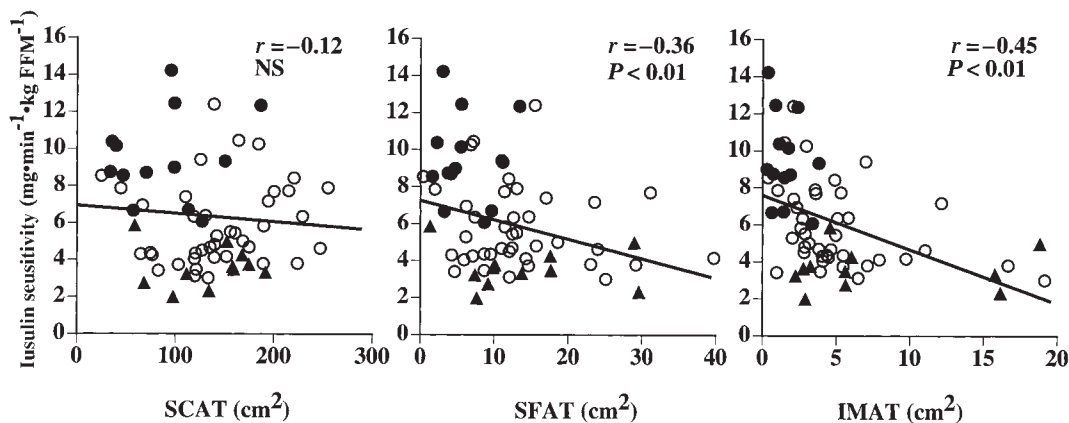
was greater than that for either SCAT or SFAT ( $P < 0.01$ ). The magnitude of reduction in these depots was similar in the obese GT and obese DM groups, although the reductions in SFAT and IMAT did not reach statistical significance in the obese DM group; this was probably because fewer DM subjects were in the weight-loss program. Neither the initial amount nor the amount of reduction in any of the thigh AT compartments predicted the magnitude of improvement in insulin sensitivity after weight loss.

Weight loss effectively increased the mean value for muscle attenuation in both the obese GT and obese DM groups ( $P < 0.01$ ) (Table 4). In addition, weight loss preferentially reduced the amount of LDM in obese GT and obese DM subjects ( $P < 0.01$ ), accounting for all of the reduction in total muscle cross-sectional area (Table 4). This suggests that the reduction in muscle cross-sectional area with weight loss was partly a function of the reduction in muscle lipid content.

#### DISCUSSION

The goal of the current study was to determine whether distinctive AT depots can be identified within the thigh and whether these are related to insulin resistance. The novel finding of our study is that the areas of AT interspersed in and around thigh muscle, which we have described as SFAT and IMAT, are greater in obesity and are significantly associated with insulin resistance. In contrast, SCAT, which comprises nearly 90% of total thigh AT and which was markedly greater in obese and type 2 DM subjects, was not associated with insulin resistance. Previous studies found that total thigh (7, 8) and lower-body (23, 24) adiposity were not associated with insulin resistance. However, these studies did not quantify the various compartments of AT as we did in the current study. Thus, it appears that there is a regional pattern of thigh AT distribution, specifically an increased intermuscular component, that is associated with insulin resistance in a manner analogous to that of AT distribution in the abdomen (4).

Previous investigations found increased amounts of AT infiltrating skeletal muscle in the elderly (25), muscular dystrophy patients (10, 26), and people with other myopathies (9, 11). The current data are the first to suggest that AT interspersed around and between skeletal muscle is associated with insulin resistance



**FIGURE 3.** Associations of subcutaneous adipose tissue (SCAT), subfascial adipose tissue (SFAT), and intermuscular adipose tissue (IMAT) with insulin sensitivity among lean glucose-tolerant (GT) subjects (●), obese GT subjects (○), and obese subjects with diabetes mellitus (DM; ▲) ( $n = 68$ ). FFM, fat-free mass.

**TABLE 4**  
Effects of weight loss (WL) on thigh adipose tissue area and skeletal muscle composition<sup>1</sup>

	Obese GT (n = 28)		Obese DM (n = 10)	
	Pre-WL	Post-WL	Pre-WL	Post-WL
SCAT (cm <sup>2</sup> )	154 ± 8	114 ± 8 <sup>2</sup>	131 ± 17	104 ± 14 <sup>2</sup>
SFAT (cm <sup>2</sup> )	15 ± 1	10 ± 1 <sup>2</sup>	15 ± 3	13 ± 2
IMAT (cm <sup>2</sup> )	6 ± 1	3 ± 1 <sup>2</sup>	9 ± 2	6 ± 2
MA (HU)	35.0 ± 0.9	37.3 ± 0.8 <sup>2</sup>	33.3 ± 1.6	37.5 ± 1.9 <sup>2</sup>
NDM (cm <sup>2</sup> )	180 ± 12	182 ± 10	175 ± 20	180 ± 26
LDM (cm <sup>2</sup> )	134 ± 10	110 ± 8 <sup>2</sup>	150 ± 22	102 ± 15 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm SE$ . GT, glucose tolerant; DM, type 2 diabetes mellitus; SCAT, subcutaneous adipose tissue; SFAT, subfascial adipose tissue; IMAT, intermuscular adipose tissue; MA, muscle attenuation; HU, Hounsfield unit; NDM, normal-density muscle; LDM, low-density muscle.

<sup>2</sup>Significantly different from pre-WL,  $P \leq 0.01$  (paired *t* test).

in obesity irrespective of sex or age. A mechanism that would explain the association between IMAT and insulin resistance has not been clearly defined. It is possible that AT interspersed in and around skeletal muscle may impair muscle blood flow, reduce insulin diffusion capacity, or increase local concentrations of fatty acids, all of which have been shown to be associated with insulin-resistant glucose metabolism in skeletal muscle (27–29). Further, it has been suggested that an increase in IMAT contributes to insulin resistance through enhanced rates of lipolysis within skeletal muscle (30).

Another finding of the current investigation was that muscle attenuation was reduced in obese GT subjects and obese subjects with type 2 DM. Reduced muscle attenuation was strongly associated with skeletal muscle insulin resistance in obesity and type 2 DM. This finding is consistent with several previous reports from our laboratory (14, 19, 20, 31, 32). This accumulation of muscle lipid most likely represents fat between the muscle fibers and within the fibers themselves, but because of the limited resolution of CT, the intracellular compared with extracellular lipid in muscle cannot be distinguished or directly measured. It is also possible that factors such as muscle water content may influence its attenuation as measured with CT, although this hypothesis has never been tested. Nevertheless, these results are in accord with others suggesting that lower muscle attenuation determined with CT represents increased lipid contained within muscle (10, 33). In addition, the current study showed that increased area of LDM was associated with insulin resistance independent of total body adiposity. These results agree with our previous finding of an association between increased lipid content in muscle and insulin resistance in obese subjects without type 2 DM (14). The current results are also in accord with investigations that found higher triacylglycerol content in skeletal muscle biopsy samples in association with insulin resistance (13). Further, we have shown that the amount of lipid contained within skeletal muscle fibers, measured with histochemical methods, is higher in obesity and in type 2 DM (34).


A methodologic issue of interest concerns the extent to which this CT method can distinguish between muscle tissue itself and AT interspersed within skeletal muscle, ie, the partition we termed IMAT. The attenuation value as measured by CT is a characteristic of the tissue density, and muscle registers higher attenuation values than does AT. The spatial resolution of the 512 × 512 image was 0.94 mm, which should permit reasonably good delineation of AT and skeletal muscle. The histograms in Figure 2, obtained in a lean volunteer and an obese volunteer,

show clear separations between adipose and muscle tissue. Moreover, the elemental composition (electrons per unit mass) also contributes to the resolution of tissue on CT so that the higher fraction of hydrogen in AT allows it to be better differentiated from other tissues (35). Further, evidence that this CT method is capable of measuring 2 distinct lipid components came from the results of multivariate regression analysis. First, our data showed that the AT interspersed between muscles and the lipid contained within muscle were both strongly associated with insulin resistance. To determine whether these thigh composition variables had independent associations with insulin sensitivity, a multivariate regression analysis including all parameters of thigh adiposity and thigh muscle composition was performed. It revealed that the amount of thigh IMAT accounted for 24% of the variance in insulin sensitivity and the amount of LDM independently explained an additional 14% of the variance. These data suggest that the IMAT and the lipid contained within muscle each have an independent association with insulin resistance.

Weight loss with reductions in both fat mass and fat-free mass improves insulin sensitivity in obesity irrespective of the presence of type 2 DM (15, 19, 36). The majority of weight change is due to loss of fat mass, and this was reconfirmed in the current study. The loss of thigh fat mass determined with DXA represented ≈5% of the total fat mass lost, whereas loss of abdominal AT accounted for 30% of the total fat mass lost during an identical weight-loss program (19). With respect to the distribution of AT in the thigh, there were decreases in subcutaneous, subfascial, and intermuscular AT components. None of the amounts by which these depots were reduced predicted the corresponding degree of improvement in insulin sensitivity. In a recent study, we observed that weight loss resulted in a decrease in visceral AT that was correlated with the improvement in insulin sensitivity, whereas the loss of total thigh AT was not (19). These results are similar to the current finding that the loss of thigh AT failed to predict the absolute improvement in insulin sensitivity. However, it is interesting to note that thigh IMAT, which had the strongest association with insulin resistance before weight loss, showed the greatest proportionate loss of all the thigh AT components during the weight-loss program.

The current study also provides new insight into the effect of weight loss on the composition of lean tissue. Before weight loss, obese subjects (GT and DM) had a greater cross-sectional area of skeletal muscle than did lean subjects. This difference was due to the greater amounts of LDM in obesity. With weight loss, muscle cross-sectional area was reduced because of a

selective decrement in LDM area while the amount of NDM remained unchanged; this led to an increase in mean muscle attenuation. Evidence suggests that fat accumulation within skeletal muscle is an important marker of insulin resistance in obesity, but the effects of weight loss on muscle lipid content or muscle composition have only been investigated recently when we reported that weight loss improves insulin sensitivity and increases muscle attenuation (19). The current findings of reduced muscle fat content because of weight reduction in both obese GT and obese DM subjects concur with these previous weight-loss studies, although the degree of alteration in muscle composition could not account for the absolute improvement in insulin sensitivity as a result of weight loss in the current study. Thus, the intervention data do not provide a clear mechanistic link between muscle lipid content and insulin sensitivity as has been found in animal models of insulin resistance (37).

In summary, the current study provided the novel observation that the distribution of AT deposition within the thigh is an important body-composition determinant of insulin resistance in obesity and type 2 DM. Specifically, accumulation of AT interspersed around and between skeletal muscle is a relatively strong marker of insulin resistance, whereas the size of the much larger thigh SCAT depot is not associated with insulin resistance. Another component of thigh adiposity, namely skeletal muscle with reduced attenuation on CT, is also associated with insulin resistance. Weight loss decreases the amount of AT infiltrating muscle in the thigh and improves insulin sensitivity in obesity and type 2 DM. Thus, compartmentalization of thigh AT into subcutaneous and intermuscular depots may be important in determining the relation between regional body composition and insulin-resistant glucose metabolism. 

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