Contributions of Total Body Fat, Abdominal Subcutaneous Adipose Tissue Compartments, and Visceral Adipose Tissue to the Metabolic Complications of Obesity

Steven R. Smith, Jennifer C. Lovejoy, Frank Greenway, Donna Ryan, Lilian deJonge, Jacques de la Bretonne, Julia Volafova, and George A. Bray

Obesity is related to the risk for developing non–insulin-dependent diabetes mellitus (NIDDM), hypertension, and cardiovascular disease. Visceral adipose tissue (VAT) has been proposed to mediate these relationships. Abdominal subcutaneous adipose tissue (SAT) is divided into 2 layers by a fascia, the fascia superficialis. Little is known about the radiologic anatomy or metabolic correlates of these depots. The objective of this study was to relate the amounts of VAT, SAT, deep subcutaneous abdominal adipose tissue (DSAT), and superficial subcutaneous abdominal adipose tissue (SSAT) to gender and the metabolic complications of obesity after adjusting for total body fat and to discuss the implications of these findings on the measurement of adipose tissue mass and adipose tissue function. The design was a cross-sectional database study set in a nutrition research center. Subjects included 199 volunteers participating in nutrition research protocols who also had computed tomography (CT) and dual energy x-ray absorptiometry (DEXA) measurement of body fat. The amount of DSAT was sexually dimorphic, with women having 51% of the subcutaneous abdominal fat in the deep layer versus 66% for men ($P < .05$). Abdominal fat compartments were compared with metabolic variables before and after adjusting for body fat measured by DEXA using 2 separate methods. The unadjusted correlation coefficients between the body fat measures, R^2, were largest for fasting insulin and triglyceride and smaller for high-density lipoprotein (HDL) cholesterol and blood pressure. A large portion of the variance of fasting insulin levels in both men and women was explained by total body fat. In both men and women, the addition of VAT and subcutaneous abdominal adipose tissue depots only slightly increased the R^2. In men, when body fat compartments were considered independently, DSAT explained a greater portion of the variance ($R^2 = .528$) in fasting insulin than VAT ($R^2 = .374$) or non-VAT, non-DSAT subcutaneous adipose tissue ($R^2 = .375$). These data suggest that total body fat is a major contributor to the metabolic sequelae of obesity, with specific fat depots, VAT, and DSAT also making significant contributions.

OBESITY IS RELATED to several metabolic disturbances such as insulin resistance, impaired insulin secretion, non–insulin-dependent diabetes mellitus (NIDDM), hypertension, dyslipidemia, and cardiovascular disease.[1,2] The metabolic risks associated with obesity are closely correlated with a central (abdominal), rather than a peripheral (gluteo-femoral) fat pattern. These complications of obesity have been attributed to increases in visceral adipose tissue (VAT) with an associated increase in portal vein free fatty acid levels.[3] The gold standard for measuring VAT is computed tomography (CT), although waist circumference is highly correlated with VAT in both men and women.[4,5] Several investigators have cast doubt on the hypothesis that VAT alone is responsible for the metabolic complications of obesity.[6,7] Abdominal subcutaneous adipose tissue (SAT) may also contribute to the metabolic syndrome.[7-11]

During the course of analyzing CT scans performed to measure VAT cross-sectional area, we observed that the SAT can be separated into a deep and superficial layer by a fascia.[12] The fascia is also visible by magnetic resonance imaging (MRI) and ultrasonography. The purpose of these studies is 2-fold. Our first aim is to describe the radiographic anatomy of the abdominal SAT as it relates to gender and adiposity. Our second aim is to relate these anatomically discrete compartments to metabolic risk factors in both men and women. Because total body fat is related to these metabolic variables, these analyses were performed without and with adjustment for total body fat.

MATERIALS AND METHODS

Population

The study population included all volunteers who completed a CT scan before participation in research protocols at the Pennington Biomedical Research Center (PBRC). The PBRC clinical database was queried for abdominal single slice CT scans between January 1, 1996 and January 1, 1998. We extracted CT scans from 199 volunteers who also had body composition determined by dual energy x-ray absorptiometry (DEXA) within 4 weeks of the CT. Individuals were categorized as having diabetes based on a positive medical history or a fasting plasma glucose level $\geq$ 126 mg/dL.[13] One individual with impaired fasting plasma glucose (FPG $>$ 110 and $<$126) was grouped with the nondiabetic individuals. Diabetics (n = 18) were excluded from the analysis of the relationship of body composition to fasting insulin, high-density lipoprotein (HDL), triglyceride, and blood pressure. One volunteer was excluded who had prior surgical abdominoplasty that distorted the SAT anatomy in question. For individuals who participated in multiple protocols, we selected the first baseline CT scan and matching DEXA. All volunteers provided written informed consent before CT and DEXA scanning. All women had a negative pregnancy test before CT scanning. Of the overall group, 131 were participating in weight loss studies and 68 were participating in nutritional studies that included only healthy volunteers. Of the women, 26 were classified as postmenopausal, and of these, 14 were taking hormone replacement therapy.

From the Pennington Biomedical Research Center, Baton Rouge; and the Baton Rouge General Health Center, Baton Rouge, LA.

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Address reprint requests to Steven R. Smith, MD, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808.

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Body Composition Analysis

Single slice CT images were acquired for each volunteer using a GE High-Speed computed tomographic scanner (GE, Milwaukee, WI). After removal of all metal clothing objects, the volunteer was placed in the supine position in the CT scanner with the arms over the head. CT images were acquired at the level of the interspace between the 4th and 5th lumbar vertebrae with a slice thickness of 10 mm at 140 kV and 340 mA. Images were stored on digital tape and transported to the PBRC for analysis on a Sun Sparc workstation (Palo Alto, CA) running the Analyze image analysis software (CNSoftware, Rochester, MN).

Anatomic placement of a representative CT scan is shown in Fig 1. The anatomy of the SAT is outlined for clarification. Total adipose tissue (TAT) was defined as the sum of adipose tissue pixels inside a line tracing the skin. VAT was segmented by drawing a line, which begins at the linea alba, bisects the rectus abdominus, the internal oblique, the iliacus, and laterally around the peritoneum surrounding the vertebral body to join at the midline anterior to the vertebral body. All pixels inside this line that met criteria for adipose tissue x-ray attenuation were counted as intraabdominal adipose tissue. All pixels outside this line are classified as SAT (TAT − VAT = SAT). For each subject, an x-ray attenuation histogram was created for both adipose tissue and skeletal muscle (psoas). This histogram was then used to determine the attenuation value for adipose tissue for each individual scan as described by Kvist et al.[14] The midpoint between the mean attenuation value for adipose tissue and the mean attenuation value for skeletal muscle was used as the upper boundary for classifying pixels as adipose tissue or other soft tissue. Houndsfeld units for the upper and lower boundary averaged −34 and −190, respectively.

Superficial subcutaneous abdominal adipose tissue (SSAT) and deep subcutaneous abdominal adipose tissue (DSAT) were separated by tracing the fascia superficialis with a mouse driven cursor and adjusted by the reader. All images were measured by a single reader. The fascia is shown by the solid arrows in Figs 1 and 2. The fascia superficialis was completely visualized in approximately 75% of the volunteers. In volunteers in which the fascia was not completely visualized, gaps in the lateral fascia were interpolated by the observer to connect visible fascia. Based on our observations using ultrasound, the fascial discontinuity is due to a CT artifact known as volume averaging rather than an anatomic interruption of the fascia superficialis (Smith SR and de Jonge L, unpublished observations). This can occur when a fascial

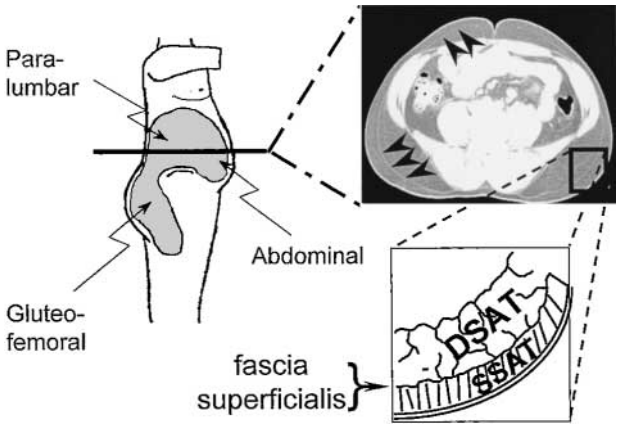


Fig 1. Location of CT scanning and demonstration of the fascia superficialis. The location of the CT scan acquisition is illustrated for clarity. The black arrowheads on the CT image mark the fascia superficialis. The fascia superficialis separates the SAT into a deep and superficial layer. The superficial layer, also known as lamellar adipose tissue, is often very thin in central obese men. The deep layer, also known as areolar adipose tissue and abbreviated as DSAT, is predominant posteriorly at the L4-L5 interspace, although the anterior deep compartment is sometimes substantial in size. Although not well illustrated in this image, the anterior and posterior DSAT compartments are contiguous. The DSAT extends superiorly at least to the inferior aspect of the ribs and inferiorly in a contiguous fashion to the lower crease of the buttocks. In some individuals, the DSAT extends inferiorly into the upper leg region. At the level of the L4-5 interspace, the posterior-lateral portion of the fascia is often penetrated by what appears to be a neurovascular bundle from the dorsal spine.

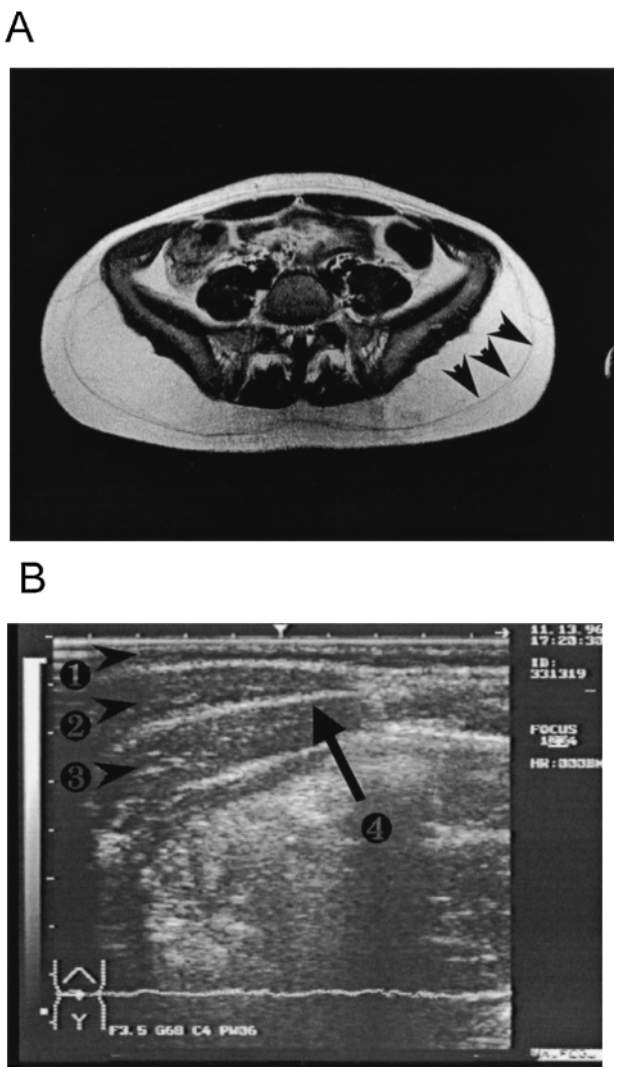


Fig 2. MRI and ultrasound of the fascia superficialis. (A) The fascia superficialis is demonstrated by MRI and annotated with arrowheads for clarity. The image was obtained at the L2-3 level using a GE 2.0 Tesla MRI scanner with the following protocol; 512 × 512 matrix, 1 NEX, spin echo with respiratory compensation, 10 mm thick, no gaps, TR350/TE 12. (B) The fascia superficialis (arrow, 4) separates the SSAT (arrowhead, 1) from the DSAT (arrowhead, 2) is clearly observed on this 2-D ultrasound of the anterior abdomen. A 5-MHz probe was used on an Aloka ultrasound. For orientation, the umbilicus is to the right and the rectus abdominus muscle is marked with an arrowhead (3). The marks across the top of the image equal 1 cm each. By ultrasound, the fascia is continuous in the anterior region. Posteriorly, the fascia is often multilayered.

plane is not perpendicular to the scan axis, resulting in loss of visualization of the fascia. The fascial discontinuity was not related to the body mass index (BMI) or gender of the volunteers. The fascia superficialis consists of a single layer in the anterior portion of the abdomen, but is commonly multilayered in the posterior SAT. The multiple layers of the posterior fascia superficialis appear as thin echogenic lines by ultrasonography (Smith SR and de Jonge L, unpublished observations). When multiple layers were present, the middle of the layers was chosen for defining the fascia superficialis. Adipose tissue pixels between the fascia superficialis and the skin were defined as SSAT. DSAT was defined as the total SAT pixels minus the superficial adipose tissue (SAT − SSAT = DSAT). The sum of pixels for each region was multiplied by the pixel size in mm^2 and divided by 100 to convert to areal measurements (mm^2) to cm^2.

Multislice CT scanning was performed on 18 additional individuals. These images were acquired using the same protocol described above, except that an additional 4 images were obtained every 5 cm above the L4-5 interspace and 2 additional images acquired 5 and 10 cm below the interspace.

Body composition was obtained on a Hologic QDR 2000 (Hologic, Waltham, MA). Body fat is represented as mass in kilograms.

Analytical Laboratory Methods

Blood samples were obtained via antecubital venipuncture. Insulin was measured using an automated microparticle enzyme immunoassay (Abbott IMX, Abbott Park, IL). HDL and triglycerides were measured using an enzymatic assay (Beckman Synchron CX5, Brea, CA). Blood pressure was measured in the sitting position using a mercury sphygmomanometer.

Statistical Analysis

Analyses were performed using Statview for Windows, version 5.0 SAS and SAS, version 6.12 (both SAS, Cary, NC). A P value $< .05$ was considered significant for all analyses. Regression plots (figures) were performed on unadjusted raw data (n = 199).

Three analyses were performed to relate the metabolic variables to the body composition variables. First, Pearson correlation was performed for each body composition variable in relationship to metabolic values from nondiabetic individuals (n = 187) using the same procedure. Insulin and triglyceride levels were log transformed before all analyses to normalize the distributions.

A second analysis was performed to "adjust" the body composition variables for the total body fat level as follows. An R^2 procedure was used to correlate percent body fat (measured by DEXA) to each metabolic variable (insulin, triglyceride, and HDL-cholesterol) and blood pressures. The residuals of this correlation analysis were then tested to determine if they contributed significantly to the model determined from the regression of the metabolic variables against body fat alone. Each model was tested for the presence of multicolinearity using several statistics. No evidence for multicolinearity was found.

The variable selection analysis is confounded by the presence of the measures of central fat (DSAT and VAT) in both the variable used to adjust (total body fat, the denominator) and the measure of interest (DSAT and VAT, the numerator). To overcome this limitation, we performed a third analysis. In this analysis, we used estimates of VAT and DSAT volumes derived from the single slice scans to generate a third variable: total body superficial subcutaneous adipose tissue (tSSAT) as follows. A 7-slice CT scanning protocol was used to measure DSAT and VAT volumes in 18 healthy men and women. This population were aged 21 to 50 (mean ± SD, 32.8 ± 8.8), with a body mass by DEXA between 39.8 and 116.2 kg (mean ± SD, 87.1 ± 19.0 kg) and body fat between 17.9 and 52.5 (mean ± SD, 35.5 ± 9.4 kg). Therefore, the body composition of these subjects is similar to the study population. The cross-sectional areas were converted into volumes using the following equation[14]:

$$\sum_{1}^{i=7} = a_i \frac{(b_i + c_i)}{2} \quad (1)$$

where a_i is the distance between scans (5 cm) and b_i and c_i are the areas of adipose tissue in 2 adjacent scans. The correlation coefficients between the single slice CT areas and the multislice volumes are shown in Table 1. Table 1 shows that the L4-5 slice is a valid location for estimation of both DSAT and VAT volume. These volumes were then converted kilograms of adipose tissue using the constant 0.9193 kg/L adipose tissue. The following regression equations were used to convert the single slice data into multislice data using the following regression equations:

$$\text{VAT(kg)} = [\text{VAT}(\text{cm}^2 \text{ at L4-5}) \times 0.038] - 0.452 \quad (2)$$

$$\text{DSAT(kg)} = [\text{DSAT}(\text{cm}^2 \text{ at L4-5}) \times 0.016] - 0.016. \quad (3)$$

These values were then subtracted from the total body fat (DEXA) to obtain an independent measure of tSSAT using the equation:

$$\text{tSSAT} = \text{total body fat(kg, DEXA)} - [\text{VAT(kg)} + \text{DSAT(kg)}] \quad (4)$$

using the regression equations 2 and 3 above.

The slopes and intercepts of regressions (Figs 3 to 6) were compared across gender using the GLM procedure (SAS).

RESULTS

Summary data for the population are shown in Table 2. The age of the men and women were similar, but in most other variables, they differed significantly. Although the men were heavier, they had less total body fat, and fat made up a smaller percentage of total weight. As expected, HDL-cholesterol was higher in the women. Triglycerides and systolic blood pressure were similar, but the diastolic blood pressure was lower in the women. A number of differences were noted in the various adipose tissue compartments, and these are analyzed in more detail below. The only one worth noting here is that there was no difference in the VAT area between men and women.

CT measured total cross-sectional adipose tissue area increased as body fat content increased in both men and women to an equal extent. VAT area was greater in men compared with women across body fat mass as has been previously described. Men and women did not differ in the slope or intercept between fat mass and total abdominal SAT.

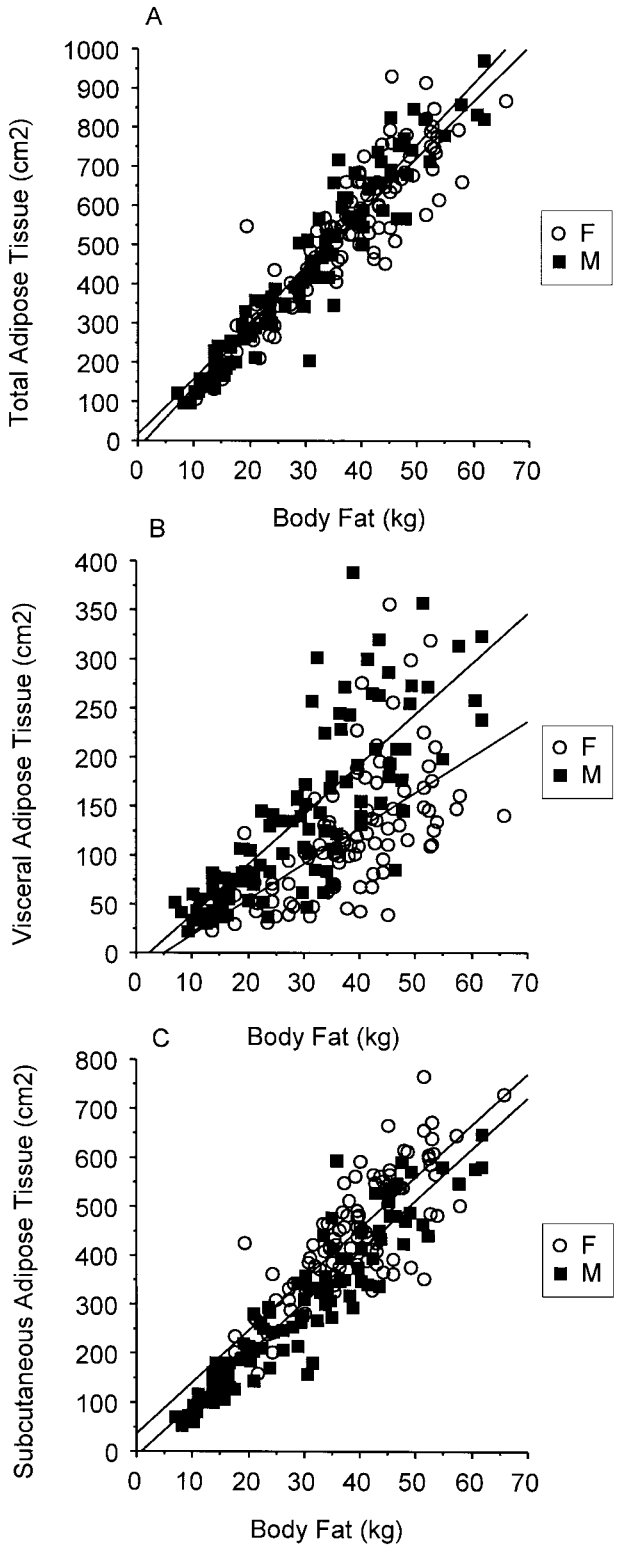
The abdominal SAT was comprised of a deep (DSAT) and

Table 1. Correlation Between Single Slice Measures (DSAT and VAT) and Volumes Measured by Multislice CT Scanning

	CT Slice						
	Pelvis −10	−5	L4,5	+5	+10	+15	Diaphragm +20
VAT	0.86	0.88	0.89	0.89	0.96	0.96	0.93
DSAT	0.02	0.46	0.88	0.84	0.70	0.04	—

NOTE. Pearson correlation coefficients were calculated for each slice regressed against total depot adipose tissue volume as described in Materials and Methods.

superficial layer (SSAT) separated by the fascia superficialis (Fig 1). The expected relationships between TAT, SAT, and VAT and body fatness were observed (Fig 3). In men, the amount of DSAT was greater than the amount of SSAT for any given level of subcutaneous fat (Fig 4A). The converse was true for SSAT, in which the women had more SSAT than men for any given amount of subcutaneous fat cross-sectional area in cm^2 (Fig 4B). Women had 50.8% DSAT on average versus 65.6% in men (insets, Fig 3). These relationships also held when DSAT and SSAT were correlated to total body fat, although the variability was somewhat greater than for total abdominal CT cross-sectional area (Fig 5).



The ratio between the amount of VAT and the total subcutaneous adipose, the V/S ratio, has been used as a description of the propensity to store fat in the visceral depot. Figure 6A (inset) shows the V/S ratio within each gender. As had been previously observed, the V/S ratio was greater for men than women, although a significant amount of overlap was seen. In a similar fashion, the ratio of the DSAT to the total amount of SAT, the D/S ratio, was greater for men than women (Fig 6B, inset). The amount of body fat did not appear to influence the V/S ratio for either men or women (Fig 6A). In contrast, the D/S ratio increased in this cross-sectional data set for men ($r^2 = .19$, $P < .001$), but not women (Fig 5B). Men with NIDDM (n = 10) when compared with the group of nondiabetics (n = 93) had higher DSAT/SAT ratios (0.714 ± 0.014 *v* 0.650 ± 0.008, $P = .01$), but not V/S ratios (0.542 ± 0.056 *v* 0.480 ± 0.024, $P = .40$, data not shown). In contrast, women with NIDDM (n = 7) when compared with the group of nondiabetics (n = 89) had higher V/S ratios (0.512 ± 0.081 *v* 0.264 ± 0.12, $P = .01$), but not DSAT/SAT ratios (0.494 ± 0.023 *v* 0.509 ± 0.009, $P = .40$, data not shown).

To determine the metabolic significance of SAT layers, we measured overnight fasted insulin, HDL cholesterol, triglycerides, and blood pressure in a subset of the original 199 men and women. The results are summarized in Table 3 for unadjusted correlation coefficients with respective *P* values. For men, the highest correlation coefficient for HDL is SSAT ($r = -.289$); for triglyceride, VAT ($r = .471$); for insulin, DSAT ($r = .779$), for SBP, VAT ($r = .203$); and for DBP, SSAT ($r = .253$). For women, the highest correlation coefficient for HDL is SSAT ($r = -.132$); for triglyceride, VAT ($r = .644$); for insulin, total body fat ($r = .521$), for SBP, VAT ($r = .334$); and for DBP, total body fat ($r = .271$).

Next, we adjusted the cross-sectional areas for total body fatness and then modeled the relationships between the adjusted fat distribution parameters and the metabolic variables. The results are presented in Table 4. For men, the models with the highest correlation coefficient for HDL, triglycerides, insulin, and blood pressure included all body fat compartments. After including body fatness in the models, VAT, DSAT, SSAT, and DSAT made small contributions. For example,

Fig 3. Relationship of body fat to CT measured abdominal adipose tissue compartments. Body fat, measured by DEXA, is correlated to the amount of (A) TAT by CT scanning, (B) VAT, or (C) SAT. Linear regression analysis provides the following equations: TAT = 14.1 body fat (kg) + 17.4 ($r^2 = .769$; F); TAT = 15.5 body fat (kg) − 19.6 ($r^2 = .918$; M) (P = NS); VAT = 3.65 body fat (kg) − 18.0 ($r^2 = .641$; F); VAT = 5.13 body fat (kg) − 11.65 ($r^2 = .769$; M), ($P < .05$*); SAT = 10.49 body fat (kg) − 35.37 ($r^2 = .698$; M); SAT = 10.38 body fat (kg) − 8.02 ($r^2 = .873$; M), (P = NS). * $P < .05$ for the comparison of slope across gender.

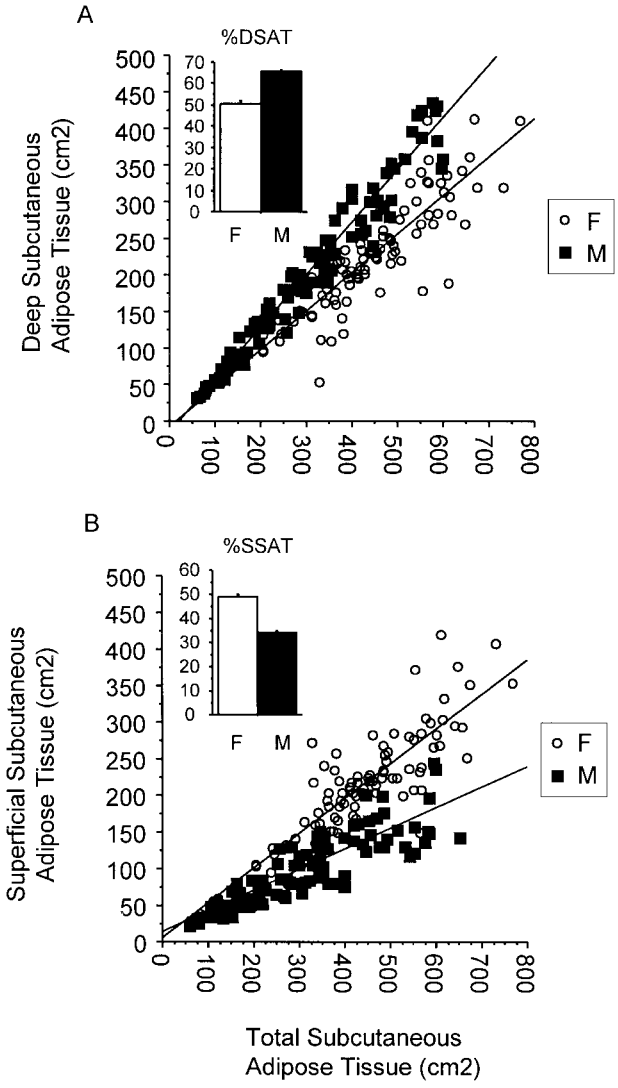


Fig 4. Relationship of DSAT and SSAT area CT to measured abdominal SAT area. The amount of SAT (cm^2) is plotted against the amount of either (A) DSAT (cm^2) or (B) SSAT (cm^2). The insets show the mean percent DSAT or percent SSAT for men and women, n = 103 and n = 96, respectively; $P < .001$ by unpaired t test. Linear regression analysis provides the following equations: DSAT (cm^2) = 0.525 SAT (cm^2) − 6.6 ($r^2 = .791$; F); DSAT (cm^2) = 0.717 SAT (cm^2) − 13.7 ($r^2 = .955$; M)*; SSAT (cm^2) = 0.475 SAT (cm^2) + 6.6 ($r^2 = .756$; F); SSAT (cm^2) = 0.283 SAT (cm^2) + 13.7 ($r^2 = .769$; M)*. * $P < .05$ for the comparison of slope across gender.

body fat alone explained 50.8% of the variance in fasting insulin in men. Adding VAT to the model increased the R^2 to 56.3, whereas adding DSAT increased the variance explained to 61.0%. The addition of VAT, DSAT, and SSAT increased the R^2 to 64.3%.

Because the measurement of total body fat includes the VAT, DSAT, and SSAT compartments, we next converted the VAT and DSAT cross-sectional areas into mass using equations 2 and 3. These masses were then subtracted from the total body fat mass to provide the non-VAT, non-DSAT body fat (tSSAT). This removes the potential concerns regarding multicollinearity from the statistical analysis. Each variable is now an independent measure of a single body fat compartment, rather than including multiple compartments. These results are presented in Table 5. For men, R^2 values for the HDL models are very low (R^2 0.051 to 0.062), and no single model is clearly superior. For triglyceride, tSSAT and VAT have the largest R^2 (R^2 = .255). The relationships between blood pressure and body fatness are modest.

In men, DSAT by itself was highly correlated to fasting insulin (R^2 = .528). The R^2 for tSSAT and VAT were lower (R^2 = .375 and .374, respectively). The addition of VAT to

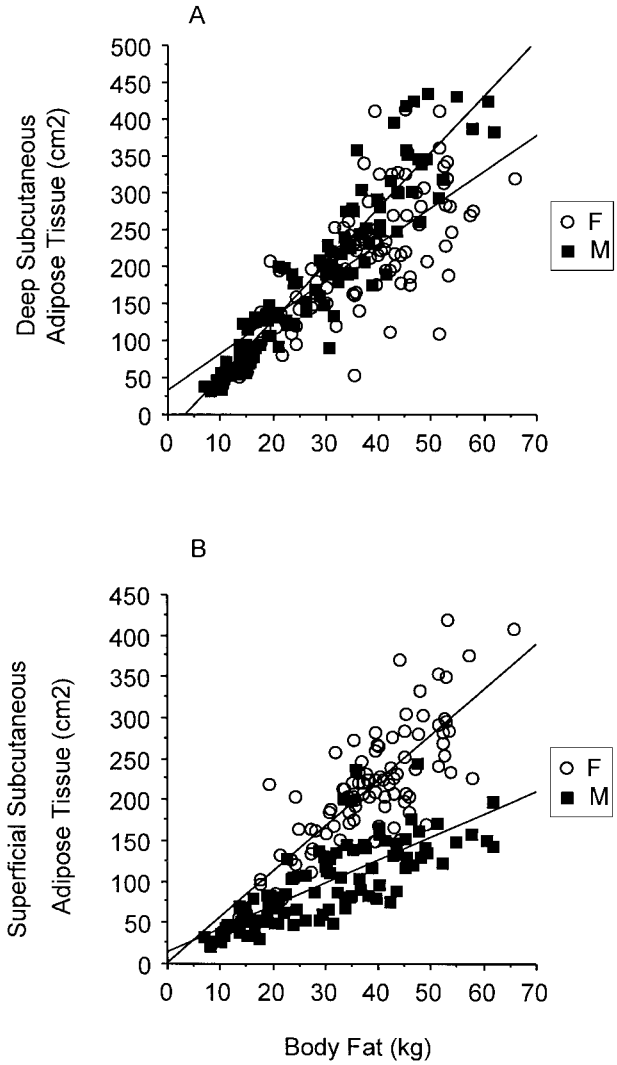


Fig 5. Relationship of DSAT and SSAT area (cm^2) to total body adipose tissue (kg). The amount of body fat measured by DEXA (kg) is plotted against the amount of either (A) DSAT (cm^2) or (B) SSAT (cm^2). $P < .001$ by comparison of slope for the regression equation. Linear regression analysis provides the following equations: DSAT (cm^2) = 4.94 body fat (kg) + 33.64 ($r^2 = .444$; F); DSAT (cm^2) = 7.60 body fat (kg) − 23.96 ($r^2 = .869$; M)*#; SSAT (cm^2) = 5.55 body fat (kg) + 1.72 ($r^2 = .656$; F); SSAT (cm^2) = 2.78 body fat (kg) + 15.9 ($r^2 = .603$; M)*. * $P < .05$ for the comparison across gender for slope. # $P < .05$ for the comparison across gender for intercept.

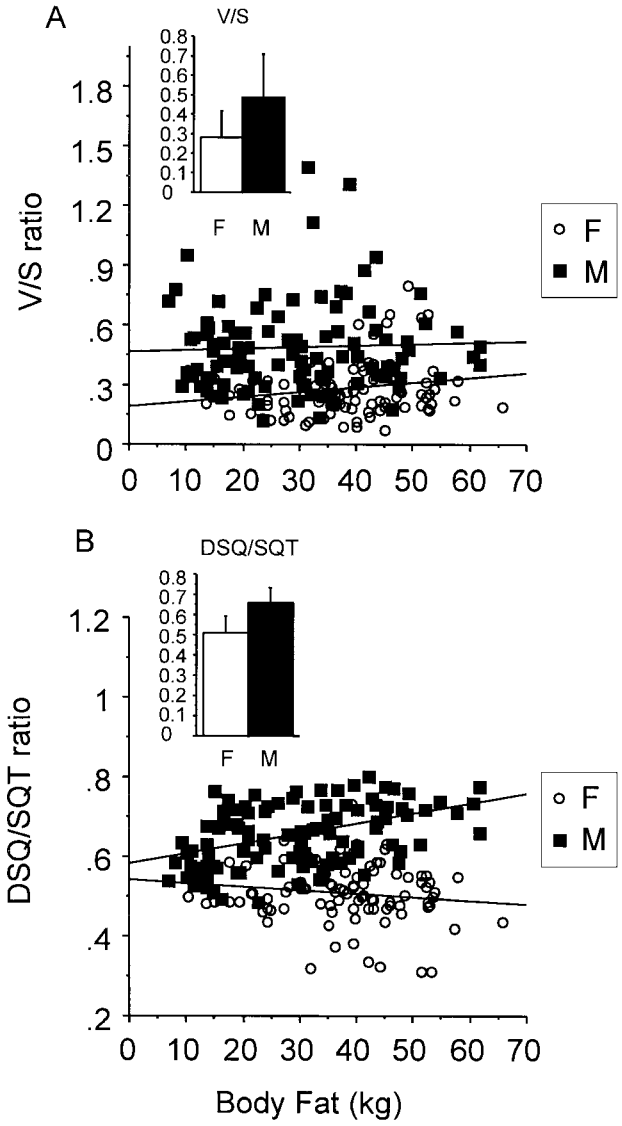


Fig 6. Relationship between the visceral to subcutaneous ratio (V/S) and the deep subcutaneous to total subcutaneous ratios (D/S) to body fat measured by DEXA. The V/S and the D/S are shown for both males and females. The slopes for the regression between body fat and V/S ratio are not significantly different from 0 for both males and females. The slope for D/S is significantly different from 0 for males, but not females (body fat by D/S interaction, $P < .001$). For males, the regression equation is D/S ratio = 0.002 body fat + 0.58, ($r^2 = .19$, $P < .001$). The inset for each figure illustrates the mean value for each ratio across gender. $P < .001$ between gender for both D/S ratio and V/S ratio.

DSAT only modestly improved the R^2 from .528 to .568, ($P = .0152$ for the comparison of the single variable model to the 2 variable model). In contrast, adding DSAT to VAT increased the variance explained from 0.374 to 0.568 ($P < .0001$).

For women, less than 2% of the variance in HDL can be explained by body fatness. The best model for triglyceride is tSSAT plus VAT ($R^2 = .286$), and the addition of DSAT provides no additional benefit in the model ($R^2 = .288$). The best model for fasting insulin is tSSAT plus VAT ($R^2 = .260$), and DSAT provides no additional benefit in the model ($R^2 = .262$).

DISCUSSION

The purpose of these studies was 2-fold. First, we aimed to describe the anatomy of these adipose tissue compartments in relation to gender and total body fatness. Second, we aimed to relate the amount of these adipose tissue depots to the metabolic complications of obesity.

A review of the anatomic literature and texts found few references to the observed SAT fascia. One of the first descriptions was found in Gray's anatomy, 1905. The fascia had been identified as the *fascia superficialis* with the more superficial adipose tissue layer being named the areolar adipose tissue and the deeper adipose tissue being named the lamellar adipose tissue.[15] We chose to retain the term *fascia superficialis*, but to substitute the more descriptive terms, deep and superficial subcutaneous adipose tissue. As the Nomina Anatomica had no listing for either lamellar or areolar adipose tissue, we felt the use of the term deep and superficial subcutaneous adipose tissue would prevent unnecessary confusion.

The advent of the surgical technique of liposuction reopened an examination of the anatomic aspects of subcutaneous fat.[16] Alexander and Dugdale[17] were the first to "rediscover" SAT layering. Using high resolution CT scanning, Johnson et al[18,19] examined abdominal adipose tissue layers in obese women. They observed significant variability in the ratio of DSAT between women and a high correlation between the DSAT area and the VAT area. In another study of adipose tissue layering in women, Markman and Barton[20] made 3 general observations. First, they found anatomic evidence for 3 general regions for accumulation of DSAT, the periumbilical region, the flanks bilaterally, and the gluteo-femoral regions. Next, they observed that the proportion of DSAT varied between women. Lastly, they suggested that the DSAT accounts for approximately 50% of the total SAT in women, a finding identical to our own.

We measured the amount of adipose tissue above and below

Table 2. Characteristics of the Study Population

	Female	Male
Age (yr)	41.5 ± 11.7	40.8 ± 13.7
Weight (kg)	82.4 ± 15.3	94.4 ± 20.1*
Body fat (kg)	38.0 ± 11.0	29.4 ± 13.7*
Fat (%)	45.2 ± 7.0	29.5 ± 8.7*
HDL (mg/dL)	50.4 ± 10.5	38.1 ± 8.1*
Insulin (μU/mL)	13.5 ± 13.8	14.1 ± 16.0
Triglycerides (mg/dL)	103.5 ± 68.8	113.9 ± 70.2
Systolic BP (mm Hg)	119.6 ± 17.5	123.3 ± 14.1
Diastolic BP (mm Hg)	77.2 ± 10.0	81.1 ± 8.0*
TAT (cm^2)	554.2 ± 177.0	435.9 ± 222.2*
VAT (cm^2)	120.5 ± 65.8	139.0 ± 87.9
SAT (cm^2)	433.8 ± 137.8	297.0 ± 152.6*
SSAT (cm^2)	212.5 ± 75.3	97.6 ± 49.2*
DSAT (cm^2)	221.2 ± 81.4	199.4 ± 112.0
V/S ratio	0.28 ± 0.14	0.49 ± 0.22*
DSAT/SAT ratio	0.51 ± 0.08	0.66 ± 0.08*

NOTE. Cell content: mean ± SD.

*Indicates significant gender difference ($P < .05$).

Table 3. Simple Correlation Between Body Composition Variables and Metabolic Variables

	Body Fat (kg)	VAT (cm^2)	DSAT (cm^2)	SSAT (cm^2)	SAT (cm^2)	HDL Cholesterol (mg/dL)	Log Triglyceride (mg/dL)	Log Insulin (μU/mL)	SBP (mm Hg)	DBP (mm Hg)
Body fat (men)*										
VAT	.805	—								
(cm^2)	<.01									
DSAT	.931	.716	—							
(cm^2)	<.01	<.01								
SSAT	.788	.516	.765	—						
(cm^2)	<.01	<.01	<.01							
SAT	.935	.689	.978	.883	—					
(cm^2)	<.01	<.01	<.01	<.01						
HDL cholesterol	−.269	−.229	−.251	−.289	−.278	—				
	.02	.04	.02	<.01	<.01					
Log triglyceride	.49	.471	.429	.346	.427	−.334	—			
	<.01	<.01	<.01	<.01	<.01	<.01				
Log insulin	.773	.705	.779	.532	.742	−.343	.491	—		
	<.01	<.01	<.01	<.01	<.01	<.01	<.01			
SBP	.15	.203	.14	.168	.156	.008	.098	.205	—	
	.16	.05	.19	.11	.14	.95	.38	.09		
DBP	.25	.243	.228	.253	.25	−.1	.113	.157	.660	—
	.02	.02	.03	.02	.02	.37	.31	.19	<.01	
Body fat (women)†										
VAT	.616	—								
(cm^2)	<.01									
DSAT	.668	.332	—							
(cm^2)	<.01	<.01								
SSAT	.812	.458	.557	—						
(cm^2)	<.01	<.01	<.01							
SAT	.835	.445	.892	.872	—					
(cm^2)	<.01	<.01	<.01	<.01						
HDL cholesterol	−.055	−.074	.042	−.132	−.048	—				
	.63	.52	.71	.24	.67					
Log triglyceride	.515	.644	.329	.424	.42	−.196	—			
	<.01	<.01	<.01	<.01	<.01	.08				
Log insulin	.521	.504	.316	.496	.445	−.287	.404	—		
	<.01	<.01	<.01	<.01	<.01	.02	<.01			
SBP	.229	.334	.189	.204	.222	−.074	.287	.173	—	
	.03	<.01	.07	.05	.03	.52	<.01	.15		
DBP	.271	.255	.199	.215	.234	−.019	.224	.236	.700	—
	<.01	<.01	.05	.04	.02	.87	.05	.05	<.01	

*Pearson correlation coefficients for metabolic variables and body composition variables. *P* values are noted below the respective *r* value. For HDL-cholesterol, n = 83; triglyceride, n = 82; insulin, n = 71; blood pressure, n = 92.

†Pearson correlation coefficients for metabolic variables and body composition variables. *P* values are noted below the respective *r* value. For HDL-cholesterol, n = 75; triglyceride, n = 75; insulin, n = 63; blood pressure, n = 88.

the fascia superficialis in 199 men and women. These studies show that the subcutaneous abdominal adipose tissue layers are sexually dimorphic, with men showing a greater proportion of DSAT (~65% for men *v* 50% for women) at any level of subcutaneous or total body fat. This sexual dimorphism is similar to the sexual dimorphism seen for VAT in relationship to the amount of subcutaneous abdominal adipose tissue.

Because of these similarities between the layering of abdominal SAT and VAT, we next compared the amount of these depots, measured by single slice CT, with the metabolic variables insulin, triglycerides, HDL-cholesterol, and blood pressure. Using simple correlation analyses, it is apparent that the body fat compartments are highly correlated to each other. In other words, as body fatness increases, VAT, and the subcutaneous compartments, DSAT and SSAT, increase. As independent variables, total fat, VAT, DSAT, and SSAT are good predictors of metabolic risk, especially fasting insulin. In general, the correlation coefficients for body fatness and metabolic risk factors are higher for men than for women. Almost all of the women in this study were premenopausal, and estrogen may provide some protection for the mechanism that links body fatness and metabolic risk. The relationships between blood pressure, HDL, and body fatness are statistically significant, but weak in comparison to triglyceride and fasting insulin. As such, the remainder of this discussion will focus on fasting insulin and body fatness.

Table 4. R^2 Values From Multiple Regression Analyses Using Cross-Sectional Areas (cm^2) Adjusted for Total Percent Body Fat by DEXA

	Log Triglyceride (mg/dL)	HDL (mg/dL)	Log Insulin (μU/mL)	Systolic (mm Hg)	Diastolic (mm Hg)
Men:					
Model 1					
Body fat (%)	.237	.061	.508	.022	.075
Model 2					
Body fat, VAT	.258	.064	.563	.042	.078
Body fat, DSAT	.237	.067	.610	.022	.075
Body fat, SSAT	.241	.084	.510	.029	.078
Body fat, SAT	.237	.078	.562	.025	.075
Model 3					
Body fat, VAT, SAT	.258	.080	.607	.044	.078
Body fat, VAT, DSAT	.258	.068	.636	.042	.079
Body fat, VAT, SSAT	.259	.093	.563	.056	.083
Body fat, DSAT, SSAT	.242	.086	.626	.029	.079
Model 4					
Body fat, VAT, DSAT, SSAT	.259	.094	.643	.057	.086
	N = 83	82	71	92	92
Women:					
Model 1					
Body fat	.143	<.0001	.237	.039	.049
Model 2					
Body fat, VAT	.354	.002	.273	.063	.085
Body fat, DSAT	.150	.012	.240	.044	.055
Body fat, SSAT	.184	.024	.294	.047	.059
Body fat, SAT	.176	.0004	.250	.049	.062
Model 3					
Body fat, VAT, SAT	.366	.002	.280	.068	.092
Body fat, VAT, DSAT	.360	.014	.276	.067	.090
Body fat, VAT, SSAT	.362	.024	.312	.066	.088
Body fat, DSAT, SSAT	.186	.042	.305	.050	.063
Model 4					
Body fat, VAT, DSAT, SSAT	.366	.042	.322	.069	.092
	N = 75	75	63	88	88

R^2 values are presented for each dependent variable (triglyceride, HDL-cholesterol, insulin, systolic blood pressure, diastolic blood pressure) after adjustment for total percent body fat. In this procedure, the residuals from regression of each body fat compartment on percent fat are used in multiple regression with metabolic variables. The R^2 variable selection procedure is used in the process of selecting optimal submodels.

Because the measures of central adiposity are intercorrelated, we chose to "adjust" the measures of central adiposity of the overall body fat mass. In the first model, we correlated VAT, DSAT, and SSAT to total body fatness and used the residuals to ask the question: "do these variables add to the overall ability of body fatness to explain blood triglyceride and insulin?" This model is presented in Table 4. When overall body fat is taken into consideration, the relationships between the central adipose tissue measures (SAT, VAT, DSAT, and SSAT) and the metabolic measures are greatly reduced. For example, body fatness explains 50.8% and 23.7% of the variance in fasting insulin for men and women, respectively. Adding VAT increases the variance explained to 56.3% and 27.3% for men and women, respectively. The increasing R^2 values suggest that an increase in the amount of DSAT and VAT have an added impact on fasting insulin levels.

One problem with this adjustment procedure is that the measures of central adipose tissue are present in the measure of total body adiposity used to adjust the data. In other words, because total body fat includes SAT and VAT, adjusting VAT for total body fat includes the variable of interest. This makes the inferences drawn from the statistical less robust and subject to criticism. In an attempt to overcome this limitation, we developed regression equations that allowed us to convert the cross-sectional CT areas of central adiposity into fat mass. These values were then subtracted from the overall fat mass measured by DEXA. This provides an independent measure of VAT mass, DSAT mass, and overall non-VAT, non-DSAT fat mass (tSSAT). This eliminates any statistical concerns surrounding collinearity, because these are independent measures.

In men, DSAT by itself was highly correlated to fasting insulin (R^2 = .528). The R^2 for tSSAT and VAT were lower (R^2 = .375 and .374, respectively). The addition of VAT to DSAT only modestly improved the R^2 from .528 to .568 (P = .0152 for the comparison of the single variable model to the multiple variable model). In other words, DSAT explained 52% of the variability in insulin values compared with VAT, which explained only 38% of the variability. This result suggests a role for DSAT in the insulin resistance seen with obesity.

The latter observation is consistent with several recent studies that suggest SAT might play a role in the pathophysiology of obesity complications, particularly insulin resistance.[8,9] The

Table 5. R^2 Values From Multiple Regression Analyses Using the Three-Compartment Model (Adjusted for % Body Fat)

	Log Triglyceride (mg/dL)	HDL (mg/dL)	Log Insulin (μU/mL)	Systolic (mm Hg)	Diastolic (mm Hg)
Men					
Model 1					
tSSAT	.180	.051	.375	.010	.055
DSAT	.158	.047	.528	.019	.057
VAT	.197	.038	.374	.038	.062
Model 2					
tSSAT, VAT	.255	.061	.504	.038	.078
tSSAT, DSAT	.193	.056	.535	.019	.066
VAT, DSAT	.225	.054	.568	.039	.076
Model 3					
tSSAT, VAT, DSAT	.255	.062	.574	.039	.080
	N = 83	82	71	92	92
Women					
Model 1					
tSSAT	.068	.001	.197	.014	.023
DSAT	.01	.011	.032	.023	.018
VAT	.258	<.0001	.102	.057	.058
Model 2					
tSSAT, VAT	.286	.001	.260	.064	.072
tSSAT, DSAT	.069	.016	.197	.029	.032
VAT, DSAT	.258	.012	.116	.069	.067
Model 3					
tSSAT, VAT, DSAT	.288	.016	.262	.072	.075
	N = 75	75	63	88	88

In this analysis, VAT and DSAT volumes were subtracted from total body fat measured by DEXA to derive the tSSAT mass in kg and then expressed in percent. R^2 values are presented for each dependent variable (triglyceride, HDL-cholesterol, insulin, systolic blood pressure, and diastolic blood pressure).

majority of DSAT is located posteriorly, which may explain the findings of Misra et al,[10] who showed that posterior abdominal SAT was a better correlate of insulin sensitivity in men than VAT. As such, abdominal SAT may somehow be different from SAT from other regions. In vivo studies of SAT support this hypothesis by showing regional differences in lipid turnover[21,22] and cell size.[23]

In addition, recent reports by Lovejoy et al[24] and Kelley et al[25] also show a relationship between DSAT and insulin resistance. For example, Kelley et al[25] using stepwise regression showed a significant independent relationship between DSAT measured by CT and insulin sensitivity as measured by the insulin clamp. In contrast to our results, a population of perimenopausal women showed a significant relationship between DSAT and fasting insulin. It has been suggested that estrogen may protect women from the negative effects of body fat on insulin sensitivity.[26] Taken together, these results suggest that SAT, specifically DSAT, has a major influence on insulin sensitivity.

In women, tSSAT was more modestly related to fasting insulin (R^2 = .197), whereas DSAT and VAT were only weakly related to fasting insulin (R^2 = .032 and .102, respectively). Adding DSAT and VAT to tSSAT increased the R^2 from .197 to .262.

How is the DSAT different from the overlying adipose tissue? In a porcine model of adipose tissue development, it was shown that the more superficial layer of adipose tissue arises from the perifollicular stromal cells. In contrast, deeper adipose tissue arises from the mesenchyme. In growing swine, the deep layer of SAT accumulates at a faster rate than the more superficial subcutaneous layer.[27] Caloric restriction in lean or genetically obese swine resulted in a greater loss of DSAT compared with the superficial layer. Mersmann and Leymaster[27] proposed the hypothesis that the SSAT serves as a "thermo-insulatory" role, whereas the deeper layer functions in a "metabolic mode." The studies of Anderson and Kaufman[28] and Hood and Allen[29] suggest differences across the fat layers in lipogenic enzyme activity. Given our data on the sexual dimorphism, as well as the differences in the embryonic origins and enzyme activity of superficial and deep adipose tissue in swine, it is possible that differences in the metabolism of the deep and the superficial layers of abdominal SAT exist in humans as well. Study of the metabolism and gene expression of adipocytes from above and below the fascia superficialis will be required to test this hypothesis.

The major determinants of VAT are gender, body fat, and age.[30,31] Numerous other factors are hypothesized to be involved in the deposition of VAT. For example, sedentary lifestyles,[32] increased activity of the hypothalamic-pituitary-adrenal axis,[33-35] decreased sympathetic nervous system activity,[36] decreased capacity for fat oxidation,[37] decreased sex steroids in men[38] and in women,[39] and birth weight[40] have all been suggested to play a role in VAT accumulation. With the exception of gender and body fat, the impact of each of these factors on DSAT is unknown.

It is also notable that the correlation between DSAT and VAT is smaller for women than men (r = .33 v .71, respectively). This suggests that the mechanisms that regulate the

relative amount of VAT and DSAT are different in women compared with men. Sex steroids and the stress steroid cortisol are candidates for the observed differences. No data exist, however, on the relationships between these factors and the correlation between VAT and DSAT mass.

Biopsy of adipose tissue is an accepted procedure for the study of adipose tissue metabolism. Depending on the depth of the biopsy needle, either the superficial or deep layer can be sampled. In central obese men, for example, the deep layer of subcutaneous fat often lies less than 2 cm below the skin (data not shown). In contrast, obese women less commonly exhibit the "thin rim" of superficial adipose tissue seen in extremely obese men with a central pattern. The differential sampling of adipose tissue from above or below the fascia could lead to variability in the results if the gene expression or metabolism is different across the subcutaneous layers. We suggest that future studies, which collect SAT through percutaneous biopsy, note the position of the tissue sample in relationship to the fascia superficialis. Indeed, the fascia admits a 14-gauge needle without discernable resistance (Smith SR, 2000, unpublished observation).

Our study is limited by the use of single slice CT to measure VAT and the SAT. The correlation between single slice CT measured VAT cross-sectional area and VAT volume is high. Similarly, the relationship between single slice CT scanning of DSAT and SSAT and directly measured DSAT/SSAT volumes are high. Future studies might avoid the issue entirely by independently measuring the mass of each adipose tissue compartment using multiple slice CT or MRI and relating the quantity of each compartment to the metabolic variable in question.

In summary, radiologic studies of abdominal SAT argue for an anatomically discrete subcutaneous depot, DSAT, which is sexually dimorphic. Total body fat was a good predictor of overall metabolic risk. DSAT was more closely related to fasting insulin in men (R^2 = .528) than VAT or tSSAT (R^2 = .334 and .375, respectively). Lastly, these results, when placed in the context of the studies by Kelley,[25] Lovejoy,[24] and Grundy,[10] suggest that the portal hypothesis, as it relates to insulin sensitivity, needs to be reexamined.

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