Subcutaneous adipocyte size and body fat distribution

Yourka D Tchoukalova, Christina Koutsari, Maksym V Karpyak, Susanne B Votruba, Eliana Wendland, and Michael D Jensen

ABSTRACT

Background: Both body fat distribution and adipocyte size are associated with metabolic abnormalities.

Objective: We defined the extent to which subcutaneous adipocyte size is related to regional fat mass and to the sizes of adipocytes in other subcutaneous depots independent of adiposity, age, and sex.

Design: Data collected from 188 women and 133 men who were 18–50 y old and who had a body mass index (in kg/m^2) of 18 to 50 were analyzed. The mean size of isolated subcutaneous abdominal, femoral, and gluteal adipocytes was measured by direct microscopy or by automated analysis of digital images. Visceral fat area was measured with computed tomography. Dual-energy X-ray absorptiometry was used to calculate adiposity.

Results: Stepwise multiple regression analyses showed that abdominal adipocyte size was associated positively with visceral and subcutaneous abdominal fat areas and negatively with lower-body fat mass as a percentage of total-body fat, after control for sex and percentage body fat. Femoral adipocyte size was related to percentage body fat (P < 0.0001), whereas gluteal adipocyte size was related to visceral fat area (P = 0.002), which suggests that these 2 lower-body fat depots are distinct. Analyses of data from a subset of volunteers (n = 99) for whom we had adipocyte size from all 3 depots showed that adipocyte size from 1 depot could be better predicted if adipocyte size from other depots were known.

Conclusions: Abdominal adipocyte size is related to body fat distribution. Adipocyte size in a person seems to be globally regulated by factors independent of variations in body fat distribution. Am J Clin Nutr 2008;87:56–63.

KEY WORDS Visceral fat, body composition, biopsy of fatty tissue, obesity

INTRODUCTION

It is well known that excess adiposity is associated with a higher risk of metabolic abnormalities, such as diabetes and atherosclerosis (1). In his classic review, Vague (2) was the first to propose that distinctions should be made according to the type of excess adiposity, because upper-body (android-type) obesity is more frequently associated with obesity-related metabolic complications than is lower-body (gynoid-type) obesity. He noted that, although body fat distribution is a sexual characteristic, both men and women can vary remarkably in android or gynoid patterns (2). Other investigators have characterized obesity on the basis of adipose tissue cellularity, using the term “hypertrophic obesity” to describe fat gain that is primarily due to increased adipocyte size and the term “hyperplastic obesity” to describe fat gain that is mediated largely by an increase in the number of fat cells with lesser degrees of adipocyte hypertrophy (3, 4). As in the case of upper-body obesity (2), a greater degree of hypertrophic obesity was noted to be associated with metabolic abnormalities (3).

The association of adipocyte size or regional fat distribution (or both) with disease risk remains a topic of interest. Mean subcutaneous adipocyte size has been positively correlated with fasting (3, 5, 6) or postglucose serum insulin concentrations (3) in obese and normal-weight adults. In normal-weight women, subcutaneous abdominal (but not femoral, gluteal, or average) adipocyte size has been positively correlated with serum insulin and triacylglycerol concentrations (7). In their extensive study of obese persons, Krotkiewski et al (8) found consistent, positive associations between subcutaneous abdominal, femoral, or gluteal adipocyte size and serum insulin concentrations. They reported that, at the same level of adiposity, persons whose abdominal adipocytes were larger than their gluteal adipocytes had higher insulin and glucose concentrations than did persons with smaller abdominal than gluteal adipocytes (8). In this same study, waist-to-hip ratio was better able to discriminate obesity complications than was abdominal adipocyte size (8). Subsequent studies confirmed that subcutaneous abdominal adipocyte size is positively associated with unfavorable metabolic indexes in both sexes, whereas femoral adipocyte size displays weaker associations or none (9–12).

An obvious question that arises relates to whether adipocyte size is independently determined in each adipose region or whether it is inherently linked among depots. Some previous studies showed that the adipocyte sizes of different subcutaneous regions are strongly correlated with each other (3, 13), whereas other studies reported weaker or no correlation between subcutaneous abdominal and gluteal-femoral adipocyte...
sizes (7, 14). The small numbers of participants in these studies may compromise the ability to draw firm conclusions. The present study addressed the question of whether adipocyte size in a given subcutaneous depot is linked to a specific fat distribution pattern and to the size of adipocytes in other depots.

**Subjects and Methods**

**Subjects**

The present study used data collected from 321 persons (188 F, 133 M) from 18 to 50 y old. Subjects were classified as normal-weight, overweight, or obese according to their body mass index (BMI; kg/m²): BMI < 25, 25–30, or > 30, respectively. Volunteers were on no medications known to affect lipid metabolism, with the exception of oral contraceptives. A complete blood count, chemistry group, and routine urine analysis were documented to be within normal range respectively. Volunteers were on no medications known to affect lipid metabolism, with the exception of oral contraceptives. A complete blood count, chemistry group, and routine urine analysis were documented to be within normal range before the study.

Written informed consent was obtained from each participant. These volunteers participated in fat metabolism studies conducted by our laboratory between 1995 and 2006, which were approved by the Institutional Review Board of the Mayo Clinic.

**Body composition measurements**

Total-body and regional fat masses were assessed with dual-energy X-ray absorptiometry (DXA) (Lunar Radiation, Madison, WI) (15). Leg fat mass was considered to be lower-body fat. A single-slice abdomen computed tomography (CT) scan at the L₁–L₂ interspace was performed to measure visceral fat area. A single-slice abdomen computed tomography (CT) scan at the L₁–L₂ interspace was performed to measure visceral fat area. Body composition measurements conducted by our laboratory between 1995 and 2006, which were approved by the Institutional Review Board of the Mayo Clinic.

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**Biopsies of adipose tissue**

Adipose tissue samples from 1–3 subcutaneous sites, depending on the protocol and tissue availability, were obtained by small-needle liposuction under sterile conditions and local anesthesia. Tissue samples for biopsy were taken from the abdominal region, two-thirds of the distance from the iliac spine to the umbilicus; the gluteal region, lower lateral quadrant of the buttock; and the femoral region, on the anterior aspect of the thigh, one- to two-thirds of the distance from the superior iliac spine to the patella. Fat tissue was immediately rinsed with saline through Nitex Nylon Fiber 250/50 (Small Parts Inc, Miami Lakes, FL) and processed for measurement of adipocyte size as described below.

**Measurement of adipocyte size**

Adipocyte size was assessed by using the approach of Di Girolamo et al (17), which involves collagenase digestion of the adipose tissue sample, separation of adipocytes by centrifugation, methylene blue staining to identify the nuclei, and measurement of the cell diameter. Briefly, samples of adipose tissue were digested in collagenase (Type II C-6885; Sigma Chemical Co, St Louis, MO) in HEPES buffer [0.1 mol HEPES/L, 0.12 mol NaCl/L, 0.05 mol KC1/L, 0.005 mol glucose/L, 1.5% (wt:vol) bovine serum albumin (BSA), and 1 mmol CaCl₂/L (pH 7.4)] at 37 °C with the use of a water bath and of shaking at 100–115 rotations/min until the digestion was nearly complete, which occurred in 20–60 min. The cell suspension formed was centrifuged for 5 min at 300 × g at room temperature. Adipocytes in the top layer were mixed uniformly with a pipette, and a 50–150-μL aliquot was added to 450 μL of 0.2% methylene blue/HEPES solution for nuclei staining and incubated for 15 min at 37 °C in the water bath. A 5- to 10-μL portion of the cell suspension was placed in each well of an 8-well Teflon-coated glass slide; the slide was coverslipped, and the cell suspension was measured optically by using a microscope (Labophot 2/2A; Nikon Inc, Melville, NY) equipped with an eyepiece having a 10-mm scale reticle at phase contrast at 100× magnification. During the procedure, precautions were taken to avoid adipocyte breakage. The inclusion of immature multilocular adipocytes was avoided by excluding cells that were <35 μm in diameter.

For studies performed before the year 2000, the diameter of 100–300 fat cells was defined by direct microscopy as previously described (17). Briefly, the adipocyte diameters were compared with the scale from the reticle and were assigned to class intervals of 7 μm, which created a histogram. The average volume of the population was calculated by using the mean (±SD) diameter obtained from the histograms and by using Goldrick’s formula. Although <300 cells [the number best thought to minimize error (17)] were measured in a small number of tissue samples, we have observed good agreement between the manual sizing methods (even measuring <300 cells) and an automated measurement approach to measuring adipocyte size on the basis of digital photographs (18). Because only a subset of the data was used for these comparisons, we explored for the potential confounding effect of the change in method in the present study by including the adipocyte size method as an independent variable in the multiple regression models. For the automated method, we measured the area of ≥300 fat cells by using an in-house program designated AdCount [renamed Cell Counting and Analysis program (CCAP)] that was written by the Biomedical Imaging Resource at Mayo Clinic (18). This program measures the area and computes the diameter and the volume of the individual fat cells, and those values are used to estimate the mean of the adipocyte diameter and volume. Adipocellular lipid weight was calculated as adipocyte volume times 0.915 (density of triolein). We designate adipocyte size as the calculated mean lipid content of the measured cells.

**Determination of regional adipocyte number**

Adipocyte number in the subcutaneous abdominal fat compartment was determined by dividing the subcutaneous abdominal fat mass by the mean abdominal subcutaneous adipocyte size. Similarly, adipocyte number in the lower-body...
compartments was determined by dividing the lower-body fat mass (determined by DXA) by the mean femoral adipocyte size.

Statistical analyses

Anthropometric and body fat distribution characteristics were compared between sexes by using 2-sample t test. We performed 3-factor analysis of variance to analyze the effect of depot, sex, and BMI category on adipocyte size and number; next, we performed pair-wise comparisons using the Tukey post hoc test for the significant interactions.

The relations between percentage body fat (%BF) or regional adiposity and regional adipocyte size were analyzed by using simple linear regression models after grouping by sex. We used the logarithmic transformation of the measurements of regional adiposity to account for the curvilinear relation with the regional adipocyte size. Analysis of covariance was used to test for differences in the slope between the sexes.

We used stepwise multiple regression analyses to evaluate the independent contributions of body fat distribution to the variance in adipocyte size. Adipocyte size values were logarithmically transformed to meet the assumptions of linear regression analysis. We included BMI, %BF (5, 19, 20), age (5, 14), sex, and the method for adipocyte size determination in the models in addition to the variables of our interest, because these factors have been shown to affect adipocyte size at the whole-body level or in specific regions. A variance inflation factor was calculated to find mutually dependent predictors. We accepted variables with a variance inflation factor of <10 into the initial model and those with a significance level of <0.05 into the final model.

We also performed stepwise multiple regression analyses with and without the addition of adipocyte size of the other depots as candidate predictors by using data from a subset of volunteers (n = 99) for whom we had measured adipocyte size data in all 3 subcutaneous depots. For example, in a model to determine independent predictors of the abdominal adipocyte size, the femoral and gluteal adipocyte sizes were included in the model as independent variables.

Statistical analyses were performed by using JMP (version 6.0.0) and SAS (version 9.1.3) software (both: SAS Institute Inc, Cary, NC). P < 0.05 denoted statistical significance. Values reported are means ± SDs unless otherwise stated. Multiple linear regression results are reported with the parameter estimate and P value for each variable.

RESULTS

As expected, we found significantly (P < 0.001) greater adipose tissue mass and greater lower-body fat mass, both in absolute and relative terms, in women than in men (Table 1). Conversely, visceral fat area assessed by CT scan was significantly (P < 0.0001) greater in men than in women, despite men’s lower total fat, which indicated that a significantly greater proportion of the men’s adipose tissue was localized intraabdominally.

Three-factor ANOVA showed no significant interaction among sex, depot, and BMI category for adipocyte size. We found significant interactions only between depot and BMI category (P < 0.05); therefore, pairwise comparisons using the Tukey post hoc test were performed on the effects of depot and BMI category on adipocyte size. The results are provided in Table 2. In persons with a BMI < 25, femoral adipocyte size was larger than either abdominal or gluteal adipocyte size, but there was no significant difference between abdominal and gluteal adipocyte size. In overweight persons (BMI 25–30), the femoral adipocytes were significantly (P = 0.022) larger than the gluteal adipocytes, but the abdominal adipocyte size did not differ significantly from the size at the other 2 sites. The sizes of adipocytes from all depots in obese persons (BMI > 30) were similar. Abdominal subcutaneous adipocyte size increased gradually with increasing BMI, whereas the sizes of the gluteal and femoral adipocytes were significantly (P < 0.05) greater only in obese subjects.

Analyses of adipocyte number showed significant effects of sex, depot, and BMI (P = 0.034) (Table 3). The numbers of adipocytes that we calculated to be present in the 2 depots increased significantly (P < 0.05) by BMI category in women, but not in men. The difference in the number of adipocytes between lower-body fat and abdominal subcutaneous fat diminished with increasing BMI in men but became more pronounced in women.

There was a significant positive association between %BF and adipocyte size (Figure 1). The relations of %BF with abdominal adipocyte size (r = 0.65, P < 0.0001 and 0.68, P < 0.0001 in women and men, respectively) and femoral adipocyte size (r =

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 188)</th>
<th>Men (n = 133)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30.8 ± 8.71</td>
<td>30.3 ± 8.8</td>
<td>0.60</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 6.0 (18.3–51.9)</td>
<td>25.0 ± 4.1 (18.9–38.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Body fat mass (% of wt)</td>
<td>36.8 ± 9.6</td>
<td>20.4 ± 7.3</td>
<td>0.000001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>27.7 ± 13.8</td>
<td>17.2 ± 9.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Visceral fat area by CT (cm²)</td>
<td>48.2 ± 50.1</td>
<td>80.0 ± 74.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>(% of CT total fat area)</td>
<td>20.2 ± 7.6</td>
<td>39.9 ± 10.8</td>
<td>0.000001</td>
</tr>
<tr>
<td>Lower-body fat mass (kg)</td>
<td>10.6 ± 5.2</td>
<td>5.7 ± 2.8</td>
<td>0.000001</td>
</tr>
<tr>
<td>(% of total fat mass)</td>
<td>39.5 ± 6.1</td>
<td>34.7 ± 4.6</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1 SD (all such values). CT, computed tomography.

2 Range in parentheses (all such values).
0.63, \( P < 0.0001 \) and \( r = 0.44; P < 0.0001 \) in men and women, respectively) were stronger than with those with gluteal adipocyte size (\( r = 0.45, P < 0.0001 \) and \( r = 0.39, P = 0.0003 \) in men and women, respectively). There was a large variability in the adipocyte size for any given \%BF. The increase in size of abdominal and femoral adipocytes as a function of %BF was significantly less in women than in men (different slopes between women and men: \( P < 0.02 \) for abdominal and \( P < 0.001 \) for femoral). In contrast, the increase in gluteal adipocyte size in relation to %BF did not differ significantly (\( P = 0.6 \)) between men and women.

The increase in abdominal adipocyte size in relation to increasing abdominal subcutaneous fat area in women (\( r = 0.67, P < 0.0001 \)) and men (\( r = 0.65, P < 0.0001 \)) is shown in Figure 2A, and Figure 2B also shows that the increase in femoral adipocyte size was positively correlated with lower-body fat mass in women (\( r = 0.41, P < 0.0001 \)) and men (\( r = 0.60, P < 0.0001 \)). For any given change in lower-body fat mass, the increase in femoral adipocyte size was significantly greater in men than in women (differences in the slopes between men and women: \( P = 0.0002 \)). In contrast, the increase in abdominal subcutaneous adipocyte size for any given change in abdominal subcutaneous fat did not differ significantly between the sexes (differences in the slopes between men and women: \( P = 0.30 \)). As evidenced by the failure of adipocyte size to completely account for the differences in regional fat mass, increases in adipocyte numbers played a variable role in the expansion of adipose tissue in leg and abdominal subcutaneous regions in men and women. For lower-body fat, the adipocyte number averaged \( \approx 5 \times 10^9 \) cells in women with 5 kg of adipose lipid, but averaged \( \approx 30 \times 10^9 \) cells in those with \( \approx 25 \) kg of adipose lipid. In contrast, the adipocyte number in men averaged \( \approx 10 \times 10^9 \) cells across the range of 2 to 13 kg of leg adipose lipid measured in our volunteers.

We investigated the associations between body composition, body fat distribution, and regional subcutaneous adipocyte size after control for age, sex, %BF, BMI, and the method used to measure adipocyte size. The best models that predicted regional adipocyte size are provided in Table 4. Visceral and subcutaneous abdominal fat areas and the %BF in the lower-body region were all independent predictors of abdominal subcutaneous adipocyte size above and beyond the variance accounted for by sex, overall %BF, and BMI. Visceral and subcutaneous abdominal fat areas were positively associated with abdominal adipocyte size, whereas the relative amount of lower-body fat mass was negatively associated with abdominal adipocyte size. This model explained 55% of the variability of the abdominal subcutaneous adipocyte size. Surprisingly, CT-assessed visceral fat area contributed to the model to predict gluteal adipocyte size independently of %BF. This model explained 26% of the variance in gluteal adipocyte size. The %BF was the only significant (\( P < 0.0001 \)) predictor of femoral adipocyte size, explaining 36% of the interindividual variability.

We used data from a subset of volunteers (\( n = 99 \)) for whom we had adipocyte size from all 3 depots to assess whether adipocyte sizes in the abdominal, gluteal, and femoral depots were related and whether knowledge of the adipocyte size in one depot increased the ability to predict adipocyte size in other depots above the predictive ability of body composition or demographic variables. Subcutaneous abdominal adipocyte size was significantly correlated with femoral (\( r = 0.61, P < 0.0001 \) in women; \( r = 0.74, P < 0.0001 \) in men) and gluteal (\( r = 0.57, P < 0.0001 \) in both men and women) adipocyte size. Correlations between gluteal and femoral adipocyte sizes were significant, but less strong (\( r = 0.33, P = 0.01 \) in women; \( r = 0.57, P < 0.0001 \) in men). In addition, we assessed whether the inclusion of adipocyte

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**Table 2** Subcutaneous abdominal, gluteal, and femoral adipocyte size by BMI category

<table>
<thead>
<tr>
<th>BMI category</th>
<th>Abdominal</th>
<th>Gluteal</th>
<th>Femoral</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>0.41 ± 0.20^b&lt;sup&gt;+&lt;/sup&gt; (199)</td>
<td>0.48 ± 0.15^b&lt;sup&gt;+&lt;/sup&gt; (103)</td>
<td>0.57 ± 0.23&lt;sup&gt;b&lt;/sup&gt; (153)</td>
</tr>
<tr>
<td>25–30</td>
<td>0.59 ± 0.23&lt;sup&gt;b&lt;/sup&gt; (55)</td>
<td>0.47 ± 0.15&lt;sup&gt;b&lt;/sup&gt; (29)</td>
<td>0.67 ± 0.26&lt;sup&gt;b&lt;/sup&gt; (32)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>0.78 ± 0.24 (65)</td>
<td>0.71 ± 0.23 (26)</td>
<td>0.83 ± 0.18&lt;sup&gt;b&lt;/sup&gt; (43)</td>
</tr>
</tbody>
</table>

* All values are \( \bar{x} \pm SD; n \) in parentheses. BMI was calculated in kg/m^2. Adipocyte size is \( \mu g \) lipid/cell. The effect of depot, sex, and BMI category on adipocyte size was analyzed by using a 3-factor ANOVA followed by Tukey post hoc test as indicated. Only the depot \( \times BMI \) interaction was statistically significant (\( P = 0.006 \)). Values with different superscript letters indicate significant differences (\( P < 0.05 \)) in the adipocyte size among depots within a BMI subgroup (eg, superscript a, b, and c in a row) and among the BMI categories within a depot (eg, superscript x, y, and z in a column).

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**Table 3** Subcutaneous abdominal and lower-body adipocyte number by sex and BMI category

<table>
<thead>
<tr>
<th>BMI category</th>
<th>Abdominal</th>
<th>Lower-body</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>11.6 ± 5.5&lt;sup&gt;a&lt;/sup&gt; (81)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.0 ± 4.0 (88)</td>
<td>1.0</td>
</tr>
<tr>
<td>25–30</td>
<td>12.2 ± 2.7&lt;sup&gt;b&lt;/sup&gt; (16)</td>
<td>18.8 ± 7.8&lt;sup&gt;b&lt;/sup&gt; (15)</td>
<td>0.009</td>
</tr>
<tr>
<td>&gt;30</td>
<td>15.7 ± 4.4&lt;sup&gt;b&lt;sup&gt;h&lt;/sup&gt; (30)</td>
<td>21.6 ± 6.5&lt;sup&gt;b&lt;/sup&gt; (35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>7.1 ± 3.2 (66)</td>
<td>10.8 ± 4.4 (65)</td>
<td>0.001</td>
</tr>
<tr>
<td>25–30</td>
<td>7.5 ± 2.5 (20)</td>
<td>12.2 ± 6.5 (17)</td>
<td>0.1</td>
</tr>
<tr>
<td>&gt;30</td>
<td>10.2 ± 6.3 (16)</td>
<td>13.9 ± 3.5 (8)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* BMI was calculated in kg/m^2. Adipocyte number is \( \times 10^9 \). The effect of depot, sex, and BMI category on abdominal and lower-body adipocyte number was analyzed by using a 3-factor ANOVA followed by Tukey post hoc test as indicated. The depot \( \times sex \times BMI \) interaction was significant (\( P = 0.034 \)). Values in a column with different superscript letters indicate significant differences in the adipocyte number among BMI subgroup in a depot and each sex group.

\( ^{2} \) Abdominal compared with lower-body adipocytes.

\( ^{a,b,c} \) Significantly different regional adipocyte numbers between men and women within a depot and BMI subgroup: \( P < 0.0001, ^{a} P < 0.01, ^{b,c} P < 0.05 \).

\( ^{d} \) \( \bar{x} \pm SD; n \) in parentheses (all such values).
size from other fat depots improves the ability to predict adipocyte size in a given depot beyond the predictive ability of sex and body composition variables. Initially, we ran stepwise multiple regression analyses without including the adipocyte size of other depots to determine to the degree to which the variances of the adipocyte sizes were predicted in this smaller group. The models

**FIGURE 1.** The relation between regional adipocyte size and percentage body fat (%BF) in men (● and —) and women (○ and - - -) assessed by simple linear regression after grouping by sex. A: Abdominal subcutaneous (SQ) adipocyte size versus %BF (men: \( r = 0.69, P < 0.0001, n = 131 \); women: \( r = 0.65, P < 0.0001, n = 189 \)). B: Gluteal adipocyte size versus %BF (men: \( r = 0.45, P < 0.0001, n = 74 \); women: \( r = 0.39, P = 0.0003, n = 84 \)). C: Femoral adipocyte size versus %BF (men: \( r = 0.63, P < 0.0001, n = 90 \); women: \( r = 0.44, P < 0.0001, n = 138 \)). Slope differences between sexes were assessed with ANCOVA. There were significant sex differences in the slopes for the abdominal (\( P = 0.02 \)) and femoral (\( P < 0.0001 \)) but not gluteal (\( P = 0.6 \)) adipocyte size.

**FIGURE 2.** The relation between regional adiposity and adipocyte size in men (● and —) and women (○ and - - -) assessed by simple linear regression (using log transformation of the measurements of regional adiposity) after grouping by sex. ANCOVA covariance was used to test for slope differences between the sexes. A: Computed tomography (CT)-assessed abdominal subcutaneous (SQ) fat area (cm²) versus abdominal SQ adipocyte size (men: \( r = 0.65 P < 0.0001, n = 115 \); women: \( r = 0.67, P < 0.0001, n = 160 \); men versus women: \( P = 0.30 \)). B: Leg fat mass (kg) versus femoral adipocyte size (men: \( r = 0.60, P < 0.0001, n = 90 \); women: \( r = 0.41, P < 0.0001, n = 138 \); men versus women \( P = 0.002 \)).
Stepwise multiple regression analysis of body composition associated with regional subcutaneous adipocyte size

Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Abdominal (n = 279)</th>
<th>Gluteal (n = 155)</th>
<th>Femoral (n = 228)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter estimate</td>
<td>P</td>
<td>Parameter estimate</td>
</tr>
<tr>
<td>Female</td>
<td>-0.22</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>Body fat (% of body wt)</td>
<td>0.034</td>
<td>&lt;0.0001</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.022</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Visceral fat area by CT (cm²)</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Subcutaneous abdominal fat area by CT (cm²)</td>
<td>0.001</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Lower-body fat (% of total fat mass)</td>
<td>-0.012</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.55</td>
<td>0.26</td>
<td>0.36</td>
</tr>
</tbody>
</table>

1 CT, computed tomography. Dependent variables were logarithmically transformed to meet the assumptions of linear regression analysis. Co-linearity diagnostics found significant co-linearity between percentage body fat and the absolute leg fat mass; therefore, leg fat mass was not included as an independent variable in the model. Independent variables included were age, sex, percentage body fat, BMI, method for measuring adipocyte size (microscopic or automated), CT visceral fat area, CT subcutaneous abdominal fat area, and relative lower-body fat mass. All variables that had a significance of P < 0.05 were left in the model.

Predicted 36%, 24%, and 19% of the variance in the abdominal, gluteal, and femoral adipocyte size, respectively. The inclusion of the regional adipocyte size of the other fat depots as independent variables (Table 5) increased the predictive strength of the models [67%, 40%, and 53% (Table 5) compared with 55%, 26%, and 36% (Table 4) for the abdominal, gluteal, and femoral variances, respectively]. Thus, the size of adipocytes of a given depot is related to the size of adipocytes from the other 2 depots within the same person, independent of other known predictors of adipocyte size. It is noteworthy that, of the fat distribution variables, only the relative amount of lower-body fat remained a strong and significant negative predictor of abdominal adipocyte size.

Discussion

The main goal of the present study was to understand whether subcutaneous adipocyte size is related to body fat distribution and to adipocyte size in other subcutaneous depots. Because increases in adipocyte number can also contribute to the accumulation of adipose tissue, we also assessed sex and regional differences in adipocyte number estimated from average adipocyte size and regional fat mass. We found that sex, %BF, and indexes of body fat distribution were independent predictors of abdominal adipocyte size, whereas only %BF alone and %BF with visceral fat were related to femoral and gluteal adipocyte size, respectively. Our findings as regards regional differences in adipocyte size were generally in keeping with previous reports (5, 7, 10, 14, 21–29). Leg adipocyte number increased with increasing BMI in women, but not in men (Table 3).

The effect of BMI and regional adiposity on adipocyte number in abdominal subcutaneous and lower-body adipose tissue differed between men and women. Normal-weight men had more leg adipocytes than abdominal subcutaneous adipocytes (Table 3). An increase in leg fat to >10 kg in women was not associated with increased adipocyte size (Figure 2B), which indicated that fat mass expanded as a result of adipocyte hyperplasia. In contrast, the men with greater amounts of leg fat had

Table 5

Stepwise multiple regression analysis of body composition and adipocyte size for subjects with available adipocyte size data from all depots

<table>
<thead>
<tr>
<th>Variable</th>
<th>In Abdominal adipocyte size (n = 98)</th>
<th>In Gluteal adipocyte size (n = 99)</th>
<th>In Femoral adipocyte size (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter estimate</td>
<td>Parameter estimate</td>
<td>Parameter estimate</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>0.171</td>
<td>0.002</td>
</tr>
<tr>
<td>Abdominal subcutaneous fat area by CT (cm²)</td>
<td>0.001</td>
<td>0.0003</td>
<td>0.014</td>
</tr>
<tr>
<td>Lower-body fat (% of total fat mass)</td>
<td>-0.014</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>In Abdominal adipocyte size</td>
<td></td>
<td>0.397</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>In Gluteal adipocyte size</td>
<td></td>
<td></td>
<td>0.228</td>
</tr>
<tr>
<td>In Femoral adipocyte size</td>
<td></td>
<td>0.514</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.67</td>
<td>0.40</td>
<td>0.53</td>
</tr>
</tbody>
</table>

1 CT, computed tomography. All adipocyte size (μg lipid/cell) values were logarithmically transformed to meet the assumptions of linear regression analysis. Independent variables included were age, sex, percentage body fat, BMI, method for measuring adipocyte size (microscopic or automated), CT-assessed visceral and subcutaneous abdominal fat areas, relative lower-body fat mass, and adipocyte size from the other 2 depots. Co-linearity diagnostics found no significant co-linearity among these independent variables. All variables that had a significance of P < 0.05 were left in the model.
larger femoral adipocytes without a concomitant increase in the leg adipocyte number, which indicated that fat mass expansion was primarily due to adipocyte hypertrophy. It is noteworthy that we did not encounter men with >15 kg leg fat in the BMI range we included. In contrast, the relation between CT-assessed subcutaneous fat and adipocyte size (Figure 2A) suggests that an expansion of abdominal fat by adipocyte hypertrophy does not fundamentally differ between women and men; in addition, women have more adipocytes than do men, and those adipocytes contribute to a larger subcutaneous fat mass in women. Unfortunately, our understanding of adipocyte proliferation is incomplete. Secreted factors from hypertrophic adipocytes are reported to enhance preadipocyte proliferation (30), and there is evidence for differences in preadipocyte differentiation between femoral and subcutaneous abdominal preadipocytes in culture (31). Much more remains to be learned about the factors that determine adipose tissue proliferation in different depots in men and women.

To the best of our knowledge, this study is the first to investigate the relation between body fat distribution and abdominal adipocyte size after control for the effects of adiposity, BMI, age, and sex. Visceral and subcutaneous abdominal fat areas assessed by CT scan and the relative amount of lower-body fat emerged as significant predictors of abdominal subcutaneous adipocyte size. The significant correlation we observed between visceral fat and abdominal subcutaneous adipocyte size is in accord with the data from previous reports (7, 11, 32–34); however, previous studies did not clarify whether these associations exist after control for adiposity. Excess visceral or truncal subcutaneous fat masses are unfavorable health markers, whereas greater amounts of lower-body fat mass may protect against adverse health consequences (12, 34–37). Our finding that abdominal subcutaneous adipocyte size is associated positively with abdominal (visceral and subcutaneous) fat areas and negatively with the relative lower-body fat mass provides a plausible explanation for the relation between fat distribution and abnormal fatty acid metabolism in obesity. We have found that most of the excess FFA in upper-body obesity arises from upper-body subcutaneous fat (38), and in vitro studies suggest that larger adipocytes have greater rates of lipolysis than do smaller adipocytes (39). It has also been reported that large abdominal adipocyte size is associated with greater metabolic risk, independent of obesity and visceral fatness (10, 40).

Our observation might also explain why lower-body fat mass appears to exert independent beneficial effects on health—ie, why persons with greater leg fat mass tend to have smaller abdominal subcutaneous adipocytes. The finding that both abdominal (visceral and subcutaneous) and leg fat masses are related to abdominal adipocyte size in opposite directions is partially consistent with the hypothesis that impaired capacity of fat storage in the subcutaneous adipose tissue leads to compensatory storage of excess fat in visceral and ectopic (ie, muscle, liver, and pancreas) depots (41). Our data show that larger quantities of fat in the lower body may be the critical factor that dictates the ability of subcutaneous adipose tissue to expand to accommodate excess fat.

By including femoral and gluteal adipocyte size in the model to predict abdominal adipocyte size, we were able to account for much larger portions of the interindividual differences in adipocyte size than if we had used only body fat characteristics. These data suggest that adipocyte size in individual persons may be somewhat globally regulated over and above differences in adiposity and body fat distribution. The correlation among the adipocyte sizes of various subcutaneous depots has been reported previously (5, 9), but the mechanisms that underlie that correlation may only be speculated on at present. Possibilities include genetic or epigenetic variation in genes encoding for regulatory factors of preadipocyte proliferation or differentiation, lipid storage, or lipolysis (or all). An example is the genetic variation in the LMNA gene encoding the nuclear envelope proteins lamin A/C that is found to be a familial trait in Pima Indians (40), a population characterized by enlarged subcutaneous abdominal adipocyte size (42). Another possibility is constitutional factors. For example, a recent study showed that the cell size of slowly dividing cells, including adipocytes, is larger with larger body size (43).

To our surprise, visceral (but not femoral) fat was positively and independently associated with gluteal adipocyte size (Table 4). The gluteal fat of obese persons has been reported to be richer in deep subcutaneous fat than in fat from the thigh region (44), which may explain our observation. It is possible that gluteal adipose tissue does not completely represent lower-body fat, and, given that this tissue bed is usually much smaller in size than that of leg fat, it may be problematic to extrapolate from gluteal adipocyte metabolism to the metabolism of lower-body fat in general. We could not detect the association between visceral fat and gluteal adipocyte size in the smaller group of observations we used to assess the possible link between adipocyte sizes in other depots (Table 5). Whether this is due to the loss of statistical power from a smaller sample size or to the association of abdominal adipocyte size and visceral fat area is not clear.

In summary, these results have important implications for our understanding of how much body fat distribution and the adipocyte size from other depots contribute to the variance in regional adipocyte size, independent of adiposity, sex, and age. Because adipocyte size is thought to relate to the regulation of adipocyte function, an understanding of the factors that influence adipocyte size should be helpful. One of the new findings of the present study is that lower-body fatness and abdominal (visceral and subcutaneous) fat are independently related to subcutaneous abdominal adipocyte size, but in different directions. This suggests that relative lower-body fat mass is an indicator of the functional storage capacity of the subcutaneous adipose tissue. A second interesting finding was that gluteal adipocyte size was not related to the lower-body fat mass but was associated with visceral fat. A third new finding is that adipocyte sizes of other subcutaneous depots are the strongest determinants of adipocyte size in any given subcutaneous depot. We interpret this finding as evidence that adipocyte size in individual persons is somewhat globally regulated, so that adipocytes from different regions are more alike than they are different, even in the face of variations in level of adiposity and body fat distribution. Finally, our data suggest that lower-body fat in men expands largely as a result of adipocyte hypertrophy, whereas the expansion of lower-body fat in women is governed by adipose tissue hyperplasia.

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writing of the manuscript; SBV: data collection and writing of the manuscript; EW: data management and data analysis; and MDJ (principal investigator): study concept and design, data interpretation, and review of the manuscript. None of the authors had any financial or personal conflict of interest.

REFERENCES