Subcutaneous Adipose Cell Size and Distribution: Relationship to Insulin Resistance and Body Fat

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Objective: Metabolic heterogeneity among obese individuals may be attributable to differences in adipose cell size. We sought to clarify this by quantifying adipose cell size distribution, body fat, and insulin-mediated glucose uptake in overweight to moderately-obese individuals.

Methods: A total of 148 healthy nondiabetic subjects with BMI 25-38 kg/m² underwent subcutaneous adipose tissue biopsies and quantification of insulin-mediated glucose uptake with steady-state plasma glucose (SSPG) concentrations during the modified insulin suppression test. Cell size distributions were obtained with Beckman Coulter Multisizer. Primary endpoints included % small adipose cells and diameter of large adipose cells. Cell-size and metabolic parameters were compared by regression for the whole group, according to insulin-resistant (IR) and insulin-sensitive (IS) subgroups, and by body fat quintile.

Results: Both large and small adipose cells were present in nearly equal proportions. Percent small cells was associated with SSPG (r = 0.26, P = 0.003). Compared to BMI-matched IS individuals, IR counterparts demonstrated fewer, but larger large adipose cells, and a greater proportion of small-to-large adipose cells. Diameter of the large adipose cells was associated with % body fat (r = 0.26, P = 0.014), female sex (r = 0.21, P = 0.036), and SSPG (r = 0.20, P = 0.012). In the highest versus lowest % body fat quintile, adipose cell size increased by only 7%, whereas adipose cell number increased by 74%.

Conclusions: Recruitment of adipose cells is required for expansion of body fat mass beyond BMI of 25 kg/m². Insulin resistance is associated with accumulation of small adipose cells and enlargement of large adipose cells. These data support the notion that impaired adipogenesis may underlie insulin resistance.

Introduction

The prevalence of overweight/obesity continues to rise in the United States and worldwide, contributing to increased risk for type 2 diabetes and cardiovascular disease. Among obese individuals, those at highest risk are those who are most insulin resistant (IR) (1). The observations that 1) individuals with similar fat mass exhibit dramatic differences in insulin sensitivity (2), and 2) among IR but not insulin-sensitive (IS) individuals, dietary weight loss improves insulin sensitivity (3) suggest that biological properties of adipose tissue, independent of fat mass per se, are important determinants of insulin resistance. In this regard, the role of adipose cell size and number is a topic of great interest. Increased body fat mass appears to be characterized by increases in both adipose cell size and number (4-6), although this is somewhat controversial. Two studies show greater number of adipose cells in early-onset but not late-onset obese adults (4,6), and no increase in adipose cell number after puberty (6). Cross-sectional studies show increase in cell size with increasing BMI until body fat mass exceeds 40 kg, when cell number is noted to increase (7). Short-term overfeeding in humans leads to increased cell size among lean subjects (8,9), but not among obese subjects, who demonstrate only an increase in number of small cells with weight gain (10). Overfeeding lean and obese mice yield similar data, showing increased adipose cell size followed by increased number (11). Thus, it seems plausible that expansion of body fat mass initially is associated with increases in cell size, but once cells reach a maximum storage threshold, further increases in body fat require an increase in adipose cell number—this has not been addressed with newer more quantitative and accurate methodologies.

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Of equal importance is the relationship between adipose cell size and insulin resistance. Studies dating back to the 1970s show a positive association between human adipose cell size and elevations in plasma glucose and insulin (10.12-14) after controlling for body fat (10). In vitro, larger adipose cells exhibit decreased insulin-mediated glucose uptake (15) and greater lipolysis (16). More recent clinical studies showed that larger adipose cell size is associated with systemic insulin resistance (17,18), family history of diabetes (19), and future development of type 2 diabetes (20). As such, hypertrophic, as opposed to hyperplastic, obesity is thought to be associated with metabolic risk.

The conclusions of all of these studies are limited by use of older methodologies that were either inaccurate, nonquantitative, or relied on the assumption that all adipose cells from a given depot were the same size. For example, estimating cell size by dividing total lipid extracted by an estimate or cell count can be dramatically influenced by inaccuracies in the cell count, and assumes all cells are the same size. Estimates from light microscopy are only accurate if an even distribution of fat cells is assumed and the cells are sectioned through their equators. We and others (4,21-24) have now clearly shown that both large and small cells exist. Using Beckman Coulter Multisizer III, we can determine the average size of mature (large) adipose cells, unfettered by the proportion of smaller cells which would lower the mean size of the total cell population. Using this improved methodology, we sought to test the hypothesis that accumulation of very small adipose cells with limited fat storage capacity and/or enlargement of already mature adipose cells would be associated with insulin resistance, and to characterize differences in adipose cell size versus number in association with % body fat in overweight/obese nondiabetic individuals.

Methods

Subjects

Subjects included 148 individuals with BMI 25-38 kg/m² who were recruited via newspaper advertisements in the San Francisco Bay Area seeking “healthy volunteers” for evaluation of insulin resistance and body fat characteristics, or from the Stanford General Surgery clinic. The goal was to minimize the BMI range in order to evaluate the independent associations of cell parameters with insulin resistance. For a secondary analysis of the cell size changes in relationship to body fat alone, however, we included an expanded subject population of 160 individuals with BMIs ranging up to 58 kg/m² (12 additional subjects with BMI > 38 kg/m²). All subjects were required to be nondiabetic, as defined by a fasting plasma glucose <126 mg/dl, and in good general health with stable weight for at least 3 months prior to study entry. Individuals with a history of eating disorder or other active psychiatric conditions, major organ disease, including cardiovascular disease, gastric bypass surgery, liposuction, recent/current use of diet medications, heavy alcohol use, or pregnant/lactating were excluded. The study was approved by the Stanford University Human Subjects Committee and the NIH/NIDDK Institutional Review Board, and all subjects gave written, informed consent.

Quantitation of insulin-mediated glucose disposal

Insulin-mediated glucose disposal was quantified by a modification (25) of the insulin suppression test as originally described and validated (26,27). Briefly, subjects were infused for 180 min with octreotide (0.27 μg/m² min) to suppress endogenous insulin secre-
pulse sizes, the data were expressed as particle diameters and displayed as histograms of counts against diameter using linear bins and a linear scale for the x-axis. Analysis of adipose cell size distribution from Multisizer graphs (Figure 1, representative example) entailed, for each subject, identification of the nadir, which was defined as the midway point between which the two cell populations were present in increased frequency. The number of adipose cells below this point represented the “% small” cells. The cells to the right of the nadir are generally normally distributed. The diameter at which the Gaussian curve peaked was defined as the “peak diameter” of the large adipose cells. Given that adipose cells are not all the same size, the use of frequency histograms according to adipose cell diameter provides more detailed and accurate quantitative analysis of adipose cell size distribution than does reliance on mean size, which is influenced by not only size but also the relative number of cells with any given diameter. An increasing number of studies, using different methodologies, have now documented a high frequency of very small adipose cells (21,23,24,31,32) with 30% or more having diameters <50 um. Whether the optimal categorization of cell subgroups is bimodal or trimodal or quadrimodal is unclear, but in our hands, the bimodal distribution is the typical curve generated and thus our preferred description of adipose cell size distribution. For additional detail on Multisizer curve profiles, the reader is referred to Refs. 31 and 32, which depict numerous individual curves and demonstrate the scope and variability in lean and overweight subjects (32), and changes with weight gain (31).

The number of total body fat cells $N$ was estimated by the following formula:

$$N = \frac{\text{[body fat mass (kg)/0.9 kg/L]}}{\text{[average volume/cell ($\mu m^3$)]}} \times 10^{-15} \text{L/} \mu \text{m}^3].$$

Average volume per cell was based on the relative number of cells per each given volume bin as represented by a cell volume histogram (generated by the Multisizer software), described by the following formula:

$$\text{Average volume per cell} = \sum 4/3 \pi (d_i/2)^3 p_i,$$

In other words, we used the sum of the volumes corresponding to bin $i$ $\times$ the relative frequency ($p_i$) of that bin (31).

### Statistical analysis

Results are presented as means ± SD. A $P$-value of $<0.05$ was considered statistically significant. Potential predictors of cell size parameters were evaluated with both univariate and multivariate (general linear regression) models with adjustment for potentially contributing/confounding variables. The multivariate models included: 1) evaluation of peak diameter as a function of BF%, sex, and SSPG; 2) evaluation of % small cells as a function of BF%, sex, and SSPG; and 3) SSPG as a function of % body fat, sex, peak diameter, and % small cells. Adjustments were made for multiple comparisons, and testing for interactions between sex and other predictors was done. To determine whether adipose cell size or number changed significantly with increasing body fat mass, adipose cell size parameters were compared in individuals in the top versus bottom sex-specific quintiles of % body fat. Quintiles of % body fat were calculated separately for females and males by rank ordering % body fat (in the expanded group of $n = 160$) and dividing into five groups with equal number of subjects in each group (i.e., quintiles). Finally, we selected the most IR and IS individuals (defined as SSPG $\geq 180$ or $< 115$ mg/dL, respectively) for comparison of peak diameter and % small cells between groups with ANCOVA, adjusting for sex and % body fat. Eliminating the mid-range SSPG subjects allows for more accurate comparison of those who are truly IR or IS, providing a supplement to correlational analyses.

### Results

One hundred forty-eight subjects met BMI and general eligibility requirements, and underwent both adipose tissue biopsy and insulin suppression test. In an attempt to obtain more pronounced differences in % body fat for a secondary analysis of adipose cell size indices in relationship to % body fat, an additional 12 subjects with BMI between 38.1 and 58 kg/m$^2$, who met general eligibility requirements but did not undergo insulin suppression test, were included in this analysis. This group numbered 160, with 100 females (BMI 32.4 ± 6.3 kg/m$^2$) and 60 males (BMI 33.1 ± 4.7 kg/m$^2$). Demographic and clinical characteristics of the main cohort ($n = 148$) are shown separately for males and females in Table 1. BMI and % body fat were normally distributed for both sexes: where mean BMI and waist circumference were significantly higher in males, and % body fat was significantly higher in females. Despite higher % body fat, females were less IR than males. As shown previously (23,31,32), adipose cell diameters were distributed bimodally, that is, with the larger cells present in a Gaussian distribution and a distinct subpopulation of small cells defined as those with a diameter below the frequency nadir. Figure 1 shows representative

| Characteristic | Males, $n = 57$ | Females, $n = 91$ | $P$-value |
|----------------|----------------|-----------------|----------|
| Age, y         | 55 ± 8         | 52 ± 10         | 0.03     |
| Ethnicity (C/B/A/H) | 44/8/2/3     | 67/5/8/11       | 0.19     |
| BMI, kg/m$^2$  | 32.0 ± 2.9     | 30.6 ± 3.3      | 0.008    |
| Body fat, %    | 35.6 ± 4.3     | 43.1 ± 4.6      | <0.001   |
| Waist circumference, cm | 109 ± 8       | 99 ± 10         | <0.001   |
| Systolic BP, mm Hg | 129 ± 14      | 122 ± 16        | 0.008    |
| Diastolic BP, mm Hg | 78 ± 8        | 70 ± 8          | 0.001    |
| Total-C, mg/dL | 180 ± 36       | 198 ± 35        | 0.004    |
| Triglyceride, mg/dL | 150 ± 90      | 109 ± 54        | 0.009    |
| HDL-C, mg/dL   | 42 ± 10        | 56 ± 17         | <0.001   |
| LDL-C, mg/dL   | 110 ± 32       | 122 ± 29        | 0.03     |
| Fasting glucose, mg/dL | 102 ± 10      | 98 ± 10         | 0.03     |
| SSPG, mg/dL    | 183 ± 70       | 160 ± 90        | 0.04     |
| % Small cells  | 53 ± 11        | 54 ± 12         | 0.47     |
| Mean diameter, $\mu m$ | 67 ± 8         | 72 ± 10         | 0.001    |
| Peak diameter, $\mu m$ | 104.9 ± 11.6  | 115.5 ± 16.2    | <0.001   |
| Cell number$^*$ | 1.3E11 ± 5.1E11 | 1.2E11 ± 1.0E11 | <0.001   |

SSPG, steady-state plasma glucose.

$^*$Kruskal-Wallis test used for non-normally distributed data.
curves for nine subjects with varied sex, BMI, and % body fat. Despite individual variability, the general pattern of two cell size sub-populations, large and small, on either side of a frequency nadir is evident. Peak diameter (center of the Gaussian) of adipose cells was significantly lower in males versus females (105 ± 14 vs. 116 ± 16 μm, \( P < 0.001 \)). There was no significant sex difference in the % small cells, but the total body number of adipose cells was significantly increased in the males.

**TABLE 2** General linear regression models predicting cell size parameters and insulin resistance (SSPG) in 148 healthy adults

| Dependent variable                | Independent variables | \( \beta \pm SE \) | Standardized \( \beta \) | \( P \)-value |
|-----------------------------------|-----------------------|-------------------|--------------------------|-------------|
| Peak diameter, μm                 | % Body fat            | 0.65 ± 0.26       | 0.26                      | 0.014       |
|                                   | Female sex            | 6.80 ± 3.22       | 0.21                      | 0.036       |
|                                   | SSPG (mg/dL)          | 0.04 ± 0.02       | 0.20                      | 0.012       |
| % Small cells                     | % Body fat            | −0.03 ± 0.21      | −0.02                     | 0.89        |
|                                   | Female sex            | 2.74 ± 2.60       | 0.11                      | 0.21        |
|                                   | SSPG (mg/dL)          | 0.04 ± 0.01       | 0.26                      | 0.003       |
| SSPG                              | % Body fat            | 2.63 ± 1.33       | 0.20                      | 0.049       |
|                                   | Female sex            | −60.44 ± 15.60    | −0.38                     | <0.001      |
|                                   | Peak diameter (μm)    | 1.06 ± 0.42       | 0.22                      | 0.012       |
|                                   | % Small cells         | 1.42 ± 0.52       | 0.21                      | 0.007       |

SSPG, steady-state plasma glucose.
Insulin resistance and adipose cell size parameters

Evaluation of the relationship between insulin resistance (SSPG) and adipose cell size parameters demonstrated modest but statistically significant relationships between SSPG and both peak diameter (Figure 2, top right) and % small cells (Figure 2, bottom right). These relationships persisted in multivariate analysis after taking into account % body fat and sex (Table 2). Percent body fat was also independently predictive of insulin resistance, and female sex was protective (Table 2). Formal testing for a sex interaction in the relationships between cell size parameters and insulin resistance was not statistically significant. Further evaluation of the relationship between adipose cell size parameters and insulin resistance included comparison of the subsets of the most IR and most IS individuals \( (n = 129) \). Results, shown in Table 3, demonstrate that the % small cells and the peak diameter of the large cells were significantly greater in the IR versus the IS subgroup, even after adjustment for sex and % body fat. Of note, when mean diameter, which includes both small and large cells, was used as an indicator of adipose cell size, no significant difference between groups was identifiable. In addition, although the calculated total body adipose cell number was similar between IR and IS subgroups, the number of large cells was significantly lower, and the ratio of small-to-large cells significantly greater in the IR subgroup.

Percent body fat and adipose cell size parameters

Analysis of the relationship between % body fat and adipose cell size parameters revealed a modest but significant direct association between % body fat and peak diameter of adipose cells (Figure 2, top left). This association persisted after adjustment for insulin

### Table 3: Adipose cell size parameters in insulin-resistant versus insulin-sensitive subgroups of overweight/moderately obese adults

|                  | Insulin sensitive \( n = 55 \) | Insulin resistant \( n = 74 \) | \( P \)-value | \( P \)-value-adj\*a |
|------------------|-------------------------------|-------------------------------|--------------|---------------------|
| % Body fat       | 40.0 ± 5.7                    | 40.0 ± 6.2                    | 0.99         | 0.09                |
| Sex (F/M)        | 39/16                         | 39/35                         | 0.03         | A                   |
| % Small cells    | 50 ± 12                       | 56 ± 11                       | 0.004        | 0.002               |
| % Large cells    | 50 ± 12                       | 44 ± 11                       | 0.004        | 0.002               |
| Peak diameter    | 109 ± 17                      | 114 ± 13                      | 0.07         | 0.01                |
| Mean diameter    | 70 ± 9                        | 71 ± 9                        | 0.47         | 0.74                |
| Total body cell numberb | 1.14E11 ± 5.55E10 | 1.17E11 ± 4.41E10 | 0.78 | 0.41 |
| Total body small cell numberb | 5.88E10 ± 2.46E10 | 6.56 ± 3.22E10 | 0.27 | 0.71 |
| Total body large cell numberb | 5.48E10 ± 2.4E10 | 4.77E10 ± 1.66E10 | 0.06 | <0.001 |
| Ratio of total small-to-large cells | 1.13 ± 0.50 | 1.46 ± 0.74 | 0.004 | 0.002 |

\*Adjusted for % body fat and sex.

bEstimated for total body based on fat mass and average cell volume.

### Table 4: Comparison of cell size and number in top and bottom quintiles of % body fat in 160 healthy adults with BMI 25-58 kg/m²

| Variable                      | Quintile 1 | Quintile 5 | Interquintile\*a difference (%) | \( P \)-value |
|-------------------------------|------------|------------|---------------------------------|--------------|
| Females \( n = 100 \)         | \( n = 20 \) | \( n = 20 \) |                                  |              |
| % Body fat                    | 38 ± 3     | 55 ± 8     | +45                             | <0.001       |
| Peak diameter                 | 113 ± 17   | 123 ± 12   | +8                              | 0.02         |
| Total body cell numberb       | 8.6E10 ± 6.4E10 | 1.5E11 ± 8.4E10 | +74                              | 0.003        |
| % Small cells                 | 53 ± 9     | 53 ± 10    | 0                               | 0.97         |
| Males \( n = 60 \)            | \( n = 12 \) | \( n = 12 \) |                                  |              |
| % Body fat                    | 30 ± 2     | 45 ± 8     | +50                             | <0.001       |
| Peak diameter                 | 105 ± 16   | 113 ± 13   | +7                              | 0.13         |
| Total body cell numberb       | 9.2E10 ± 2.3E10 | 1.6E11 ± 3.4E10 | +74                              | <0.001       |
| % Small cells                 | 49 ± 16    | 54 ± 7     | +10                             | 0.41         |

\*Interquintile difference is % difference between % body fat quintiles 1 and 5.

\*Estimated for total body based on fat mass and weighted cell volume.
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Discussion

The results of this study, which utilized a quantitative measurement of both insulin-mediated glucose disposal and adipose cell size distribution among healthy overweight to moderately obese individuals, demonstrate that insulin resistance is associated with both increased proportion of small adipose cells and increased size of large adipose cells independent of % body fat and sex (Figure 2, Table 2). Comparison of the most IR to most IS subjects, as shown in Table 3, further depicts these relationships, showing an increase in the % small adipose cells, and the diameter of the large adipose cells in the most IR versus IS subgroups. Calculated number of total body large and small cells revealed a significant increase in the ratio of small-to-large cells in the IR versus IS subgroup, which was primarily due to a decrease in the absolute number of large adipose cells. Thus, it appears that insulin resistance is characterized by increased size but decreased number of large adipose cells, after adjustments for sex and % body fat. This is the largest study to date to examine both in vivo and % body fat, the peak number of adipose cells increased dramatically with increased % body fat. The current results also extend prior reports describing an association between adipose cell size and insulin resistance. Specifically, after adjustment for % body fat and sex, we have shown that the size of the large adipose cells is directly associated with insulin resistance (Figure 2, Table 2). Results corroborate reports from multiple studies that examined insulin-mediated glucose uptake in isolated adipose cells (18) or correlated mean adipose cell size with various measures of insulin resistance in vivo (37-39). Prior studies used cruder methods and relied on mean adipose cell size, and most included a range of lean to obese individuals, increasing possibility of confounding by BMI or body fat. In a prior publication from our group, we did not see an association between peak diameter and insulin resistance in cohort of moderately obese healthy individuals. This study was small, however, and there was not a trend toward larger peak diameter in the IR group (119 vs. 115 μm), which in the context of the current findings appears to have been due to lack of statistical power. Another study using similar methodology to the current analysis demonstrated that obese type 2 diabetics had many “large” cells than similarly obese nondiabetics, but fewer “small” cells (34). Thus, the results of this study add to accumulating data suggesting that enlargement of large adipose cells occurs in association with insulin resistance independent of BMI, perhaps as a result of impaired fat uptake in small adipose cells as described.

Finally, the current results demonstrate important relationships between peak adipose cell diameter and number in relationship to % body fat. Not surprisingly, peak diameter is significantly related to increased body fat (Figure 2, Table 2), which has been previously shown with older (4-7) and newer methodology (34) in populations with wide-ranging BMIs. In the current analysis, restricted to those with BMI 25 kg/m² and above, however, this association is quite modest. Indeed, as shown in Table 4, among individuals with BMI ranging from 25 to 58 kg/m², comparison of the top and bottom quintiles of % body fat shows that for a 50% increase in % body fat, the peak diameter of adipose cells increases only by 7%, whereas the adipose cell number increases by 74%. These data
indicate that it is unlikely that increases in cell size alone can account for the increased fat storage capacity required with increasing body fat mass once individuals have exceeded normal body weight, and that increase in adipose cell number is an important component of fat storage when BMI exceeds 25 kg/m². Whether this entails recruitment of existing preadipocytes or true proliferation of cells is not ascertainable from this study and further research should address this question.

Strengths of this study include the relatively large size, with quantitative methods for measuring both insulin resistance and adipose cell size distribution, and the relative homogeneity of subjects with regard to general health status and BMI range. Limitations of this study include the estimate of total body cell number from subcutaneous abdominal adipose tissue biopsy only. It has been shown that adipose cells differ in size not only within a given depot but also between depots, which can lead to differing estimates of total body cell number (4). To minimize this limitation, we have estimated the total cell number consistently for all subjects, so the comparisons between subjects should be valid. Second, our subject sample is largely Caucasian, and thus our conclusions may not extrapolate to other ethnic groups. We also did not determine the age at which obesity began and are thus unable to differentiate between early-onset versus late-onset obesity. Although large adipose cells might be subject to breakage, we did not perform collagenase digestion and immediately fixed cells in osmium, which prevents breakage. Furthermore, there are no published data or theoretical reason to believe that breakage would occur differentially according to insulin resistance group. Thus, the associations detected with insulin resistance in this study are unlikely to be affected by large adipose cell breakage. Finally, our data show a somewhat higher proportion of small adipose cells (50%) than shown by some investigators (30%) (21,34). We cannot rule out the possibility that some of the very small cells are lipid fragments or cell debris. However, numerous other studies using a variety of techniques have found substantial numbers of small cells (21,23,24,32,34). That we find statistically significant relationships between the proportion of small-to-large cells with insulin resistance and inflammation (33), and that separation of small from large adipose cells (22,40) yields differential gene expression according to cell size, make it likely that the quantitative estimate of small cells is biologically meaningful, despite the possibility of some nondifferential background “noise.”

In conclusion, the results of this study demonstrate, in a sizable cohort of carefully selected and metabolically well-characterized overweight/moderately obese adults, that: 1) accumulation of small relative to large adipose cells, both % and absolute number, is associated with insulin resistance; 2) peak diameter of adipose cells is associated with insulin resistance and % body fat; and 3) among overweight to morbidly obese individuals, increases in % body fat are associated with relatively small increases in adipose cell size and require recruitment of additional adipose cells for triglyceride storage. Together these findings suggest that with expanding body fat mass, impairment in the ability to generate mature “large” triglyceride-storing adipose leads to accumulation of small adipose cells and is associated with insulin resistance. Although these findings provide support for the hypothesis that impaired adipogenesis/fat storage in subcutaneous adipose tissue may contribute to systemic insulin resistance, further studies are needed to delineate the underlying mechanisms regulating triglyceride storage capacity, adipogenesis, and whether impaired terminal differentiation/triglyceride uptake in subcutaneous adipose cells is causally related to systemic insulin resistance.

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