Effects of weight gain and weight loss on regional fat distribution¹–⁴

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ABSTRACT

Background: Normal-weight adults gain lower-body fat via adipocyte hyperplasia and upper-body subcutaneous (UBSQ) fat via adipocyte hypertrophy. 

Objectives: We investigated whether regional fat loss mirrors fat gain and whether the loss of lower-body fat is attributed to decreased adipocyte number or size.

Design: We assessed UBSQ, lower-body, and visceral fat gains and losses in response to overfeeding and underfeeding in 23 normal-weight adults (15 men) by using dual-energy X-ray absorptiometry and abdominal computed tomography scans. Participants gained ~5% of weight in 8 wk and lost ~80% of gained fat in 8 wk. We measured abdominal subcutaneous and femoral adipocyte sizes and numbers after weight gain and loss.

Results: Volunteers gained 3.1 ± 2.1 (mean ± SD) kg body fat with overfeeding and lost 2.4 ± 1.7 kg body fat with underfeeding. Although UBSQ and visceral fat gains were completely reversed after 8 wk of underfeeding, lower-body fat had not yet returned to baseline values. Abdominal and femoral adipocyte sizes, but not numbers, decreased with weight loss. Decreases in abdominal adipocyte size and UBSQ fat mass were correlated (r = 0.76, P = 0.001), as were decreases in femoral adipocyte size and lower-body fat (r = 0.49, P = 0.05).

Conclusions: UBSQ and visceral fat increase and decrease proportionately with a short-term weight gain and loss, whereas a gain of lower-body fat does not relate to the loss of lower-body fat. The loss of lower-body fat is attributed to a reduced fat cell size, but not number, which may result in long-term increases in fat cell numbers. Am J Clin Nutr 2012;96:229–33.

INTRODUCTION

Excess body fat is associated with dysfunctional metabolism and increased cardiovascular burden (1); however, fat distribution is as important as fat quantity for some complications of obesity (2). Excess abdominal fat contributes to dyslipidemia, hypertension, and insulin resistance (3). In contrast, the relative excess of lower-body fat is associated with a reduced cardiovascular risk profile (2). Thus, the determination of whether humans preferentially increase or decrease fat in particular depots during energy surpluses or deficits is of great importance. To test how adipose tissue prioritizes regional gains and losses of fat stores, we measured changes in regional fat masses in a longitudinal study of weight gain and loss in healthy normal-weight adults.

Adipocytes in different depots are inherently distinct in terms of their ability to store (4, 5) and release (6) fatty acids; even the characteristics of resident preadipocytes vary between depots (7, 8). Recently, we reported that, in response to overfeeding in normal-weight adults, subcutaneous abdominal adipocytes accommodate energy surplus by hypertrophy, whereas femoral adipose tissue increases fat storage by hyperplasia (9). In the current article, we report how upper-body subcutaneous (UBSQ)⁵ and lower-body subcutaneous adipose tissue responds to energy restriction and fat loss after an antecedent fat gain.

SUBJECTS AND METHODS

Subjects

We recruited 23 volunteers (15 men and 8 women) with a baseline BMI (in kg/m²) of 23.6 ± 3.9 and aged 30 ± 6 y from the community. Study volunteers were sedentary, nonsmoking, free of chronic disease, and taking no medications on a regular basis. Pregnant and postmenopausal women were not included. Women were allowed to participate during any phase of their menstrual cycle. The studies were conducted in the Mayo Clinic Center for Translational Science Activities Clinical Research Unit. The research protocol was approved by the Institutional Review Board at the Mayo Clinic, and written informed consent was obtained from all volunteers. The studies were initiated in 2003.

Protocol

Volunteers worked closely with an experienced dietitian for an initial 3-d period to assess their individual energy intakes required for weight maintenance and to ensure a constant macronutrient composition of the diet (40% carbohydrates, 40% fat, 25% protein) during the study protocol. All volunteers gained weight by using 30% of their estimated energy expenditure (EE) for 8 wk and lost weight by using 60% of their EE for 8 wk. The consumed food was delivered to the participants every day for consumption during specified periods. Weight was measured daily to the nearest 0.1 kg. The EE was assessed using indirect calorimetry, and a 3-d dietary recall was performed every 2 wk to ensure good adherence. To minimize any potential confounding by EE, volunteers were given the same absolute EE values in both weight-gain and weight-loss phases (1.2 × basal metabolic rate [BMR] for men and 1.1 × BMR for women) to ensure a constant macronutrient composition of the diet (40% carbohydrates, 40% fat, 25% protein) throughout the study. Each participant was also given the same diet, which was based on the Dietary Guidelines for Americans (10). All food was delivered to the participants every day for consumption during specified periods. In addition, the EE was assessed using indirect calorimetry, and a 3-d dietary recall was performed every 2 wk to ensure good adherence. To minimize any potential confounding by EE, volunteers were given the same absolute EE values in both weight-gain and weight-loss phases (1.2 × basal metabolic rate [BMR] for men and 1.1 × BMR for women) to ensure a constant macronutrient composition of the diet (40% carbohydrates, 40% fat, 25% protein) throughout the study.
and 20% protein) as previously described (10, 11). After the baseline period, volunteers underwent a controlled weight-gain intervention over an 8-wk interval, during which they consumed 1–3 supplements/d (400–1200 extra kcal) in addition to their usual intakes, which resulted in an ~5% gain of body weight. Supplements consisted of a choice of ice-cream shake (402 kcal; 40% fat), chocolate bars (a king-size Snickers bar, 510 kcal; Mars Inc), and energy drink (Boost Plus, 360 kcal/8 oz; Nestle Nutrition). During this time, volunteers were weighed ≥5 d/wk to enable dietitians to adjust the calorie intakes of volunteers on a daily basis. After the weight gain and testing, volunteers underwent a controlled weight-loss intervention over an 8-wk interval with the goal of allowing them to return to their baseline weights. During this time, dietitians provided counseling to support volunteers as they resumed normal eating patterns. Volunteers were also encouraged to increase their activities to aid weight loss. Body weight was monitored ≥5 d/wk with additional follow-up provided if needed; none of the volunteers expressed concern about being able to lose weight at the expected rate in response to questioning.

Body composition was assessed at baseline, halfway through the overfeeding interval, at the completion of overfeeding, halfway through the weight loss, and after weight loss. Adipose tissue biopsies were obtained from the abdominal subcutaneous region ~5 cm lateral to the umbilicus and from the anterior-lateral femoral subcutaneous region at the mid thigh at baseline, after weight gain, and after weight loss. Fat biopsies were obtained by using a modified needle aspiration under sterile conditions and with local anesthesia. Abdominal subcutaneous adipose tissue was collected at baseline, after weight gain, and after weight loss in 21 volunteers. Femoral fat biopsy was obtained at baseline, after weight gain, and after weight loss in 22 volunteers.

**Fat cell size and number**

Adipose tissue obtained from abdominal and femoral sites was collagenase digested (type II; Sigma) for 60 min at 37°C, and the resulting isolated adipocytes were photographed for fat cell sizing as previously described (13). Because of the difficulty of collecting sufficient tissue for cell size measures in some volunteers, we were able to measure both abdominal and femoral fat cell sizes at baseline, after weight gain, and after weight loss in only 12 volunteers; abdominal adipocyte size after weight gain and after weight loss in only 16 volunteers; and femoral adipocyte size after weight gain and after weight loss in only 17 volunteers. To estimate the number of adipocytes in UBSQ and lower-body fat, we divided the regional fat mass (total micrograms of lipids per depot) by the average adipocyte size (micrograms of lipids per cell) to calculate the adipocyte cell number (adipocytes per depot). This approach assumed that the vast majority of lipids in UBSQ and leg are in adipose tissue (9).

**Statistical analysis and power**

Data were analyzed with JMP 9.0.1 software (SAS Institute Inc). Continuous variables were recorded as means ± SDs. Each measurement during baseline, weight gain, and weight loss was compared by using paired Wilcoxon’s signed-rank test. Changes in the regional fat distribution and fat cell size during weight gain and weight loss were compared by using Spearman’s correlation. P < 0.05 was considered significant for all tests.

The decision of how much weight gain to induce was made by using data on the precision and accuracy of the body-composition measurements and the ethics and difficulty of working with volunteers to gain fat weight. We previously documented that the DXA equipment used at the Mayo Clinic can detect the addition of 600 g abdominal fat in a volunteer with 22.8 kg total body fat with excellent accuracy; the experimental change in abdominal fat was 600 g, and the measured increase by using DXA was 577 g (11). We showed that visceral fat measured by the combination of single-slice abdominal CT and single abdominal DXA agreed with whole-abdomen CT with a median absolute difference of ~177 mL in a population with 1961 ± 2120 mL visceral fat (12). For this study, we targeted better accuracy and precision by obtaining 3 abdominal CT slices and duplicate abdominal DXA scans. Our goal was to be able to measure the anticipated increases in regional fat mass with >90% accuracy. The observed gains in regional fat (Table 1) were within our targets for this degree of accuracy, with the possible exception of the change in visceral fat mass. Changes in fat mass (in kg) for each compartment were compared between baseline, weight-gain, and weight-loss intervals.

### RESULTS

#### Effects of weight gain and weight loss on body composition and body fat distribution

Participants gained 3.7 ± 1.4 kg during the weight-gain period and lost 2.8 ± 1.6 kg during the weight-loss period. These weight changes resulted from changes in total body fat; the fat-free mass did not change significantly throughout the study. At

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change in weight gain</th>
<th>Change in weight loss</th>
<th>Spearman’s ρ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower-body fat mass (kg)</td>
<td>0.9 ± 0.7</td>
<td>−0.5 ± 0.6</td>
<td>−0.31</td>
<td>0.16</td>
</tr>
<tr>
<td>Visceral fat mass (kg)</td>
<td>0.4 ± 0.7</td>
<td>−0.2 ± 1.5</td>
<td>−0.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UBSQ fat mass (kg)</td>
<td>1.9 ± 1.3</td>
<td>−1.7 ± 1.3</td>
<td>−0.66</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*All values are means ± SDs. Change in weight gain: difference between after weight gain and baseline; change in weight loss: difference between after weight loss and after weight gain. P values were calculated by using Spearman’s correlation analysis.*

*UBSQ, upper-body subcutaneous.*
the time of the post–weight-loss measurements, participants had not yet completely returned to baseline starting weights.

We showed significant differences in lower-body fat mass but not in visceral or UBSQ fat between baseline and weight-loss measurements (Table 2). On average, participants gained 1.9 ± 1.3 kg UBSQ fat (61% of total fat gain), 0.9 ± 0.7 kg lower-body fat (27% of total fat gain), and 0.4 ± 0.7 kg visceral fat (12% of total fat gain). During weight loss, participants lost 1.7 ± 0.6 kg lower-body fat, and 0.4 ± 0.6 kg visceral fat.

To test whether fat depots that expand with weight gain are the same ones that contract with weight loss within an individual, we tested relations between absolute changes in regional fat mass during weight gain and weight loss (Table 1). Increases in visceral and UBSQ fat mass during weight gain correlated with decreases in fat mass during weight loss. Furthermore, on average, participants lost all of the upper-body fat mass that they had gained. Although our study was not powered to detect sex differences in fat distribution during weight gain and weight loss, no sex differences in fat distributions during weight gain and weight loss were detected in our study participants.

Effects of weight loss on adipocyte size and number

On the basis of previous data (9), we did not have a sufficient number of observations to provide an adequate statistical power to determine whether relations between changes in fat cell size and regional fat gains were significant. Therefore, we restricted the analysis of fat cell size to the weight-loss portion of the study (n = 16 paired observations for the abdomen and n = 17 paired observation for the femoral). Changes in abdominal and femoral subcutaneous adipose tissue cellularity in response to body fat loss are provided in Table 3.

On average, the size but not the number of abdominal subcutaneous adipocytes decreased significantly in response to weight loss. Relative reductions in abdominal subcutaneous adipocyte size during weight loss correlated with relative decreases in upper-body fat mass (r = 0.76; P = 0.0007) (Figure 1A), which suggested that fat loss was largely attributable to decreases in adipocyte size. Changes in abdominal subcutaneous adipocyte size with weight loss were heterogeneous in men and women and did not correlate with adipocyte size observed after weight gain (Figure 2A).

Femoral adipocyte size also decreased in response to weight loss (P = 0.023), such that the leg adipocyte numbers remained stable despite the loss of 0.5 kg lower-body fat. In women but not men, weight loss consistently reduced femoral adipocyte size (−0.187 ± 0.150 compared with −0.107 ± 0.226 µg lipid/cell, respectively). As with abdominal subcutaneous adipose tissue, decreases in femoral adipocyte size correlated with decreases in lower-body fat mass (P = 0.49, P = 0.05), which indicated that fat loss resulted from decreases in femoral adipocyte size (Figure 1B). Similar to that reported during weight gain (9), the femoral adipocyte size after weight gain predicted changes in femoral adipocyte size with weight loss (P = −0.55, P = 0.023) (Figure 2B).

DISCUSSION

We previously reported that weight gains in normal-weight, healthy adults result in abdominal adipocyte hypertrophy and lower-

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T2–T1</th>
<th>T3–T1</th>
<th>T3–T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body weight (kg)</td>
<td>72.2±15.1</td>
<td>75.9±15.7</td>
<td>73±15.1</td>
<td>—</td>
<td>0.02</td>
<td>&lt;0.0001</td>
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<tr>
<td>Total body fat mass (kg)</td>
<td>21.4±8.3</td>
<td>24.5±8.9</td>
<td>22.1±9.1</td>
<td>—</td>
<td>0.06</td>
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<tr>
<td>Total fat-free mass (kg)</td>
<td>47.8±10.4</td>
<td>48.3±10.6</td>
<td>47.9±10.2</td>
<td>0.23</td>
<td>0.95</td>
<td>0.08</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>30.5±8.5</td>
<td>33.3±8.7</td>
<td>31.1±9.3</td>
<td>&lt;0.0001</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower-body fat mass (kg)</td>
<td>9.3±3.5</td>
<td>10.2±3.7</td>
<td>9.6±3.8</td>
<td>&lt;0.0001</td>
<td>0.02</td>
<td>0.0002</td>
</tr>
<tr>
<td>Visceral fat mass (kg)</td>
<td>2.0±1.3</td>
<td>2.4±1.2</td>
<td>2.2±1.9</td>
<td>0.001</td>
<td>0.84</td>
<td>0.0002</td>
</tr>
<tr>
<td>UBSQ fat mass (kg)</td>
<td>10.1±4.4</td>
<td>12.0±4.9</td>
<td>10.3±4.6</td>
<td>&lt;0.0001</td>
<td>0.26</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. P values were calculated by using the paired Wilcoxon’s signed-rank test. P < 0.05 was considered significant. Because total body weight and total body fat mass were not random variables from T1 to T2, they were not subjected to statistical testing. T1, baseline; T2, after weight gain; T3, after weight loss; UBSQ, upper-body subcutaneous.
body adipocyte hyperplasia (9), but to our knowledge, whether these newly gained lower-body adipocytes regress with weight loss was unknown. To understand the biology of regional adipose tissue responses to weight loss, we measured changes in fat-depot mass and subcutaneous adipocyte size in volunteers who lost weight through underfeeding after an experimentally induced weight gain. We showed that visceral and UBSQ fat masses had returned to preoverfeeding amounts despite an incomplete loss of gained fat, whereas lower-body fat had not returned to the preoverfeeding amount. Of equally great interest, the lower-body fat cell number did not decrease with weight loss. To our knowledge, this is the first longitudinal study to examine changes in cellularity and regional fat distribution with fat gain and loss in humans.

The finding that UBSQ and visceral fat return to pre-weight-gain amounts more rapidly than does lower-body fat during weight loss may be a reflection of differential triglyceride storage capacity or lipolysis rates of lower- and upper-body adipocytes. Although UBSQ adipocytes have a greater storage affinity for meal triglycerides than do lower-body adipocytes (4, 5, 14), they are also generally more responsive to lipolytic stimuli than lower-body adipocytes are (6, 15–17). These differences may explain why UBSQ and visceral adipose tissue more readily surrenders excess energy storage as fat than does lower-body fat. Of note, the preferential mobilization of UBSQ fat before visceral fat depots during weight loss has also been observed in obese adults (18–21). Although we could not measure visceral adipocyte size, it was still important to accurately measure visceral fat mass and, therefore, UBSQ mass at each time point. Only by doing so were we able to understand the relation between changes in abdominal fat cell size and changes in UBSQ mass.

We have shown that UBSQ and lower-body subcutaneous fat respond differently at the cellular level to accommodate increased energy storage (9). In the current article, we extended these findings to the fate of regional adipocyte behavior in response to weight loss. Our finding that decreases in UBSQ and lower-body subcutaneous fat mass during weight loss were attributed to decreases in fat cell size, but not fat cell number, was consistent with the observations that weight loss in obese adults is attributed to a reduced fat cell size without changes in the fat cell number (22–24). After weight loss, obese people have smaller adipocytes than do people with a similar percentage of body fat and stable weight. However, it is unknown whether those obese adults had more adipocytes before they became obese; some investigators have suggested that obese individuals must have developed their entire compliment of adipocytes before reaching adulthood (25). Nonetheless, evidence has suggested that postobese adults have a larger number of fat cells (23), which could be a long-term consequence of adult weight gain on adipose tissue cellularity.

One possible consequence of having a greater number of smaller adipocytes is a relative leptin insufficiency (23, 26, 27). Plasma leptin concentrations are related to body fat content and adipocyte size, and data suggested that smaller adipocytes after weight loss secrete less leptin for a given fat mass. This effect might create a dysregulation of appetite (28) and, thereby, promote excess food intake relative to energy needs. If true, our

**FIGURE 1.** Relations between relative changes in abdominal and femoral fat cell size with regional changes in fat mass. Squares represent data from men, and circles represent data from women. A: Changes in abdominal sq fat cell size with weight loss correlated with changes in upper-body fat mass ($r = 0.76, P = 0.0007; n = 16$). B: Changes in femoral fat cell size with weight loss correlated with lower-body fat mass ($r = 0.49, P = 0.05; n = 17$). Correlations were determined by using Spearman’s correlation analysis. sq, subcutaneous.

**FIGURE 2.** Relations between T2 adipocyte size and changes in adipocyte size in response to weight loss in abdominal (A) and femoral (B) fat depots. Squares represent data from men, and circles represent data from women. The relation was not significant in abdominal sq fat cells ($r = -0.07, P = 0.8; n = 16$) but was significant in femoral fat cells ($r = -0.55, P = 0.023; n = 17$) as determined by using Spearman’s correlation analysis. sq, subcutaneous; T2, after weight gain.
results suggest that modest weight gains and losses in normal-weight adults may have long-term consequence on adipose tissue cellularity, a relative reduction in leptin, increased appetite, and, hence, tendencies toward weight regain. However, a 5-y follow-up report of identical twins who had been experimentally overfed concluded that the weight gains seen were not different from those attributable to usual aging (29). A strength of the current study was its unique longitudinal design, which allowed for the evaluation of differential changes in regional fat depots with both weight gain and weight loss in the same participant. Another strength was that regional fat gains were large relative to the precision and accuracy of our body-composition measures, with the possible exception of visceral fat. We suspect, however, that the extra care we took to estimate visceral fat (ie, 3 abdominal CT slices and duplicate DXA scans) was sufficient to achieve the precision and accuracy we needed because the changes in visceral fat (as well as in UBSQ fat) during weight gain and loss were highly correlated. In contrast, changes in lower-body fat during weight gain and loss were not correlated, and this measure is more robust. Potential limitations of the study included the intentional weight gain and weight loss over a modest short duration, which may not have reflected gradual and more dramatic changes in body fat in the obese population.

In conclusion, fat gain and fat loss are more plastic in upper-body fat depots than in lower-body fat. The findings that lower-body fat does not respond to short-term reductions in nutrient intakes as robustly as UBSQ and visceral fat, and the lower-body adipocyte number does not decrease with weight loss, have implications for how we view the consequences of weight cycling. Our results imply that modest weight fluctuations in healthy adults lead to an overall increased number of fat cells, which could promote future weight gain and make weight maintenance more difficult.

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The authors’ responsibilities were as follows—VKS and MDJ: designed the research; AR-C, FHS-K, SP, and DED: conducted the research; PS and MDJ: analyzed the data; PS, MDJ, and VKS: wrote and had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES