Changes in Subcutaneous Fat Cell Volume and Insulin Sensitivity After Weight Loss

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OBJECTIVE

Large subcutaneous fat cells associate with insulin resistance and high risk of developing type 2 diabetes. We investigated if changes in fat cell volume and fat mass correlate with improvements in the metabolic risk profile after bariatric surgery in obese patients.

RESEARCH DESIGN AND METHODS

Fat cell volume and number were measured in abdominal subcutaneous adipose tissue in 62 obese women before and 2 years after Roux-en-Y gastric bypass (RYGB). Regional body fat mass by dual-energy X-ray absorptiometry; insulin sensitivity by hyperinsulinemic-euglycemic clamp; and plasma glucose, insulin, and lipid profile were assessed.

RESULTS

RYGB decreased body weight by 33%, which was accompanied by decreased adipocyte volume but not number. Fat mass in the measured regions decreased and all metabolic parameters were improved after RYGB ($P < 0.0001$). Whereas reduced subcutaneous fat cell size correlated strongly with improved insulin sensitivity ($P = 0.0057$), regional changes in fat mass did not, except for a weak correlation between changes in visceral fat mass, insulin sensitivity, and triglycerides. The curve-linear relationship between fat cell size and fat mass was altered after weight loss ($P = 0.03$).

CONCLUSIONS

After bariatric surgery in obese women, a reduction in subcutaneous fat cell volume associates more strongly with improvement of insulin sensitivity than fat mass reduction per se. An altered relationship between adipocyte size and fat mass may be important for improving insulin sensitivity after weight loss. Fat cell size reduction could constitute a target to improve insulin sensitivity.

Obesity is associated with insulin resistance and dyslipidemia and also with a very high risk of developing type 2 diabetes. Interestingly, studies of bariatric surgery (i.e., techniques that reduce or bypass the stomach in order to achieve weight reduction) show, first, that there is no clear quantitative relationship between the weight loss induced by various surgical procedures and the degree of normalization in insulin sensitivity and other metabolic parameters and, second, that metabolism is markedly improved before any significant weight loss is achieved (1,2). In a hallmark study by Klein et al. (3), a large amount of subcutaneous abdominal adipose tissue was removed from obese subjects by liposuction. This was not accompanied by

1Department of Medicine, Karolinska Institutet, Stockholm, Sweden
2Department of Surgery, Karolinska Institutet, Ersta Hospital, Stockholm, Sweden
3Department of Medicine, Karolinska Institutet, Ersta Hospital, Stockholm, Sweden
4Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden
5Department of Surgery, Södertälje Hospital, Södertälje, Sweden
6Department of Surgery, Karolinska Institutet, Stockholm, Sweden

Corresponding author: Peter Arner, peter.ner@ki.se.

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any metabolic improvement at short-
(10–12 weeks) (3) or long-term (82–208
weeks) follow-up (4). However, the
regional distribution of adipose tissue could
play a role since visceral fat mass
displays a much stronger association
with insulin resistance than the quantita-
tively much larger mass of subcutaneous
adipose tissue (reviewed in Lebovitz and
Banerji [5]). Furthermore, a study of
weight loss induced by diet/lifestyle
intervention showed that a reduction in
visceral but not subcutaneous fat mass
 correlates with metabolic improve-
ment (6). On the other hand, several
studies have shown that removal of a
subset of visceral adipose tissue (the
greater omentum) in connection with
bariatric surgery does not cause addi-
tional improvements in the metabolic
profile (7–9).

Still, factors other than fat mass could
play a role for the beneficial effects of
weight loss. Adipose tissue can expand
by increasing the number and/or vol-
ume of its fat cells, causing distinct adi-
pose morphologies termed hyperplasia
(many small fat cells), hypertrophy (few
large fat cells), or an intermediate of the
two, as previously reviewed (10). Fat
morphology can be estimated by com-
paring body fat mass with average fat
cell size. When this is performed in a
large set of individuals with a broad
range in fat mass, this results in a
curve-linear relationship (11). Values for
adipocyte size and total fat mass from an individual are then plotted
into the graph, and values above the
mean curve fit indicate hypertrophy
and those below suggest hyperplasia
(11). It is well established that large sub-
cutaneous fat cells are associated with
a pernicious metabolic profile (12), and
more recent studies demonstrate that
enlarged fat cell size increases the fu-
ture risk of developing type 2 diabetes
(13,14). Regional variations in adipocyte
size may have different pathophysiol-
ical impact; thus, enlarged fat cells in the
visceral and subcutaneous depots asso-
ciate with dyslipidemia and insulin resis-
tance, respectively (15,16). It should be
emphasized that although it is well es-
tablished that weight loss is accompa-
nied by a decrease in fat cell size, fat
cell number is not influenced by (even
pronounced) weight loss (17).

In this study, we aimed to investigate
the long-term effects of changes in fat
cell size and fat mass in different regions
on insulin sensitivity and other meta-
bolic parameters after weight loss in-
duced by Roux-en-Y gastric bypass
surgery (RYGB). Many obese patients
undergoing RYGB have concomitant
type 2 diabetes. Since insulin sensitivity
is a continuous variable and our aim was
to study improvements in insulin resis-
tance rather than impact on diabetes
prevention, subjects with type 2 dia-
betes were also enrolled.

**RESEARCH DESIGN AND METHODS**

**Subjects**

Sixty-two women undergoing surgical
 treatment for obesity were included
(cohort 1). Patients with type 2 diabetes
who were not on insulin therapy were
included. The subjects had not been in-
structed to follow any hypocaloric diet
prior to the first examination and all had
been weight stable (weight change <2
kg) for at least 1 year before their first
visit. After RYGB, subjects reported their
actual body weight every 6 months post-
operatively and were reexamined when
they reached a new weight-stable level,
which occurred on average after 24
months (14–54 months). To obtain a
sufficiently reliable relationship be-
tween fat mass and fat cell volume, par-
cularly for the baseline estimates,
inclusion of 62 subjects with a large in-
terindividual variation in BMI (20–62
kg/m²) was required (cohort 2). All of
these additionally added women were
otherwise healthy and free of continu-
ous medication and none had under-
gone any important weight reduction.

**Examinations and Calculations**

The women reported to the laboratory in
the morning after an overnight fast.
Height and weight were determined for
assessment of BMI. Systolic and diastolic
blood pressure were determined in the
supine position after 15 min of rest
with a fully automatic device (Omor
M10-IT; Omron Health Care, Hoofddorp,
the Netherlands). A venous blood sample
was obtained, and plasma levels of insu-
lin, glucose, triglycerides, cholesterol, and
HDL cholesterol were determined as
previously described (15). Low-density
cholesterol was calculated with the
Friedewald formula (18). Total body fat,
adominal (android) fat, hip (gynoid) fat,
and android/gynoid fat ratio were mea-
sured by dual-energy X-ray absorptiometry
(DXA) using a GE Lunar iDXA with the
software enCORE (version 14.10.022)
provided by the manufacturer (GE
Healthcare, Madison, WI) (19). The soft-
ware was also used to calculate esti-
mated visceral fat (EVAT) in the android
region from the following formula: total
adipose fat mass in the android region =
EVAT + estimated subcutaneous adipose
tissue (ESAT) in the android region, as
previously described (20). Determina-
tion of EVAT with this method shows a
strong correlation ($r^2 = 0.95$) with mea-
sures using computed tomography (20).
Assessment of android fat mass by DXA
is widely used and well accepted as a
valid measure. Since total android fat
mass and EVAT are used to determine
ESAT and both are valid measures, it fol-
lows that the calculation of ESAT should
also be valid. Estimated subcutaneous
adipose tissue in the android region
(ESAT) was therefore calculated as total
android fat minus EVAT.

A subcutaneous fat biopsy was
obtained from the abdominal wall at
the same level as the measured ESAT.
Fat cell weight and volume of fat cells
were determined as previously de-
scribed (15). In brief, fat cells were iso-
lated, and the diameter of 100 cells was
measured. Using established formulas
(21), the mean fat cell volume and
weight were determined. The number of
fat cells in the ESAT region was deter-
mined by dividing ESAT weight with mean
fat cell weight. After 45
min of rest, the women underwent a
hyperinsulinemic-euglycemic clamp
as previously described (15). In brief,
after an intravenous bolus dose of insulin
(1.6 units/m² body surface area) (11.1
pmol/m²), insulin was infused intrave-
nously at 0.12 units/m²/min (0.83
pmol/m²/min) for 2 h, and a variable in-
travenous infusion of glucose (200
mg/mL) was used to maintain euglycemia
between 4.5 and 5.5 mmol/L (81–99
mg/dL). The infusion rate of glucose dur-
ing the last 60 min of the clamp, when
insulin levels are in a steady state, was
used to calculate whole-body glucose
disposal rates ($M$ value). The average
values of blood glucose and insulin at
steady state during clamp were 5.05 ±
0.19 mmol/L and 1,680 ± 530 pmol/L
at baseline and 5.10 ± 0.18 mmol/L
and 1,170 ± 290 pmol/L at follow-up.

The two insulin levels differed signifi-
cantly ($P < 0.001$ by paired Student
The relationship between fat mass and fat cell volume was determined as follows: a curve fit of the relationship between ESAT adipocyte volume and measured fat cell volume was determined as previously described (11,17). The relationship is \( V = (a \times m)/(1 + b \times m) \), where \( V \) is mean fat cell volume, \( m \) is the amount of adipose tissue, and \( a \) and \( b \) are variables that are obtained by fitting the formula to subject data. The difference between measured fat cell volume and the expected fat cell volume obtained from the mean curve fit at the corresponding fat mass is indicative of adipose morphology, as previously discussed in detail (11). If the measured fat cell volume is larger than expected, adipose hypertrophy prevails, whereas the opposite is valid for hyperplasia.

Statistics

Group values are mean ± SD in text and Table 1 and ±SEM in Fig. 1. Results were compared by paired Student t test or ANCOVA and Fisher post hoc test. Differences in adipose and metabolic parameters between baseline and re-examination were calculated and compared using Spearman correlation and multiple regression analyses. Differences between curve-linear relationships were tested using Student t test as described in the supplement of a previous study (17). A P value <0.05 was considered to be statistically significant in all analyses.

RESULTS

Sixty-two obese women were included in the study comparing results before and after weight reduction, and the clinical characteristics at baseline and at follow-up are shown in Table 1. Six women had type 2 diabetes; two were treated with lifestyle intervention only, three with metformin, and one with metformin plus glibenclamide. At the follow-up
Fat Cell Size and Insulin Resistance

corresponded to a total weight loss of 42.7 mmHg. BMI decreased from an initial...

Table 2—Relationship between changes in adipose and metabolic parameters after bariatric surgery

<table>
<thead>
<tr>
<th>Adipose variable</th>
<th>( M ) value</th>
<th>Insulin</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous fat cell volume</td>
<td>0.0057</td>
<td>0.430</td>
<td>0.389</td>
<td>0.072</td>
<td>0.025</td>
<td>0.085</td>
<td>0.0021</td>
</tr>
<tr>
<td>ESAT</td>
<td>0.313</td>
<td>0.398</td>
<td>0.554</td>
<td>0.855</td>
<td>0.311</td>
<td>0.233</td>
<td>0.180</td>
</tr>
<tr>
<td>Android fat mass</td>
<td>0.060</td>
<td>0.411</td>
<td>0.701</td>
<td>0.11</td>
<td>0.152</td>
<td>0.666</td>
<td>0.063</td>
</tr>
<tr>
<td>Gynoid fat mass</td>
<td>0.372</td>
<td>0.138</td>
<td>0.752</td>
<td>0.791</td>
<td>0.021</td>
<td>0.497</td>
<td>0.0074</td>
</tr>
<tr>
<td>Android fat/gynoid fat ratio</td>
<td>0.091</td>
<td>0.038</td>
<td>0.648</td>
<td>0.121</td>
<td>0.0073</td>
<td>0.697</td>
<td>0.0013</td>
</tr>
<tr>
<td>EVAT</td>
<td>0.0154</td>
<td>0.525</td>
<td>0.438</td>
<td>0.033</td>
<td>0.085</td>
<td>0.299</td>
<td>0.027</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>0.061</td>
<td>0.151</td>
<td>0.733</td>
<td>0.186</td>
<td>0.044</td>
<td>0.710</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Values are \( P \) values following Spearman correlation test.

Table 2—Relationship between changes in adipose and metabolic parameters after bariatric surgery

Figure 2—Relationship between changes in mean fat cell volume and insulin sensitivity (\( M \) values) after gastric bypass of obese women. A: The relationship between absolute values. B: The relationship when women were divided into tertiles based on fat cell volume (FCV) changes. See Table 2 and text for statistical results.

Figure 2—Relationship between changes in mean fat cell volume and insulin sensitivity (\( M \) values) after gastric bypass of obese women. A: The relationship between absolute values. B: The relationship when women were divided into tertiles based on fat cell volume (FCV) changes. See Table 2 and text for statistical results.

examination, none of the women with diabetest were taking any antidiabetic drugs. At baseline, 20 women were treated with antihypertensive medication, and 7 additional patients had a systolic blood pressure \( \geq 140 \) mmHg. At follow-up, 13 women were still on antihypertensive medication, and 8 who had preoperatively diagnosed hypertension still had a systolic blood pressure \( \geq 140 \) mmHg. BMI decreased from an initial 42.7 ± 4.4 to 28.5 ± 4.2 kg/m\(^2\), which corresponded to a total weight loss of 33 ± 9%. The fat mass in all examined regions (ESAT, EVAT, android, etc.) decreased markedly after RYGB. Subcutaneous fat cell volumes decreased markedly after surgery, whereas there was no significant change in the number of fat cells. Not surprisingly, there was a significant improvement of the metabolic risk profile (clinical chemistry and body fat distribution) after surgery.

The distribution of fat cell volumes before and after RYGB is detailed in Fig. 1. Although a uniform distribution was observed at both examinations, the distribution curve at re-examination was markedly shifted to the left, indicating smaller cell volumes.

As expected, there was a strong relationship between the decrease in subcutaneous fat cell volume and the decrease in ESAT (\( P = 0.0012 \)). The changes in adipose parameters were compared with changes in metabolic risk factor parameters (Table 2). Adipocyte volume reduction was significantly and strongly associated with an improvement in the \( M \) value (\( P = 0.0057 \) for relationship in Fig. 2A), whereas a decrease in ESAT or total fat mass was not (\( P = 0.31 \) and \( P = 0.06 \), respectively). Assuming that changes in adipose tissue and metabolic parameters were normally distributed, multiple regression was performed and showed a significant correlation between changes in subcutaneous fat cell volume and \( M \) value independently of the initial fat cell volume (partial \( r = 0.38; P = 0.031 \)). Similar analyses with changes in \( M \) value as dependent factor and fat cell volume changes as one independent factor and fat mass changes in different depots (total fat, android, gynoid, EVAT, or ESAT) as a second independent factor only showed a significant correlation between changes in fat cell volume and \( M \) value (Table 3). We also subdivided the subjects into tertiles for changes in fat cell volume after RYGB (Fig. 2B). Changes in \( M \) values for the three groups were compared by ANCOVA using changes in ESAT as covariate. An overall effect of fat cell volume reduction was observed (\( F = 4.0; P = 0.025 \)). The mean increase in \( M \) value after bariatric surgery between women in the lowest and highest tertile of fat cell volume change was 2.0 ± 1.1 mg glucose/kg body weight/min and 3.4 ± 1.7 mg glucose/kg body weight/min, respectively (\( P = 0.0054 \) by post hoc test).

Changes in fat cell volume after RYGB were also significantly related to changes in plasma total and LDL cholesterol (Table 2). Some fat mass and fat cell volume measures correlated with individual values or blood pressure, but the associations were not consistent for any of the fat parameters (values not shown). As several patients were on antihypertensive treatment or used lipid-lowering drugs, the findings on blood pressure and different cholesterol measures were not further analyzed. In order to estimate the influence of multiple testing in Table 2, Bonferroni correction of \( P \) values was performed (\( P \) multiplied with 7, which was the number of dependent variables for each independent variable). Only subcutaneous fat cell volume versus \( M \) value remained statistically significant (\( P = 0.04 \)).

The relationship between mean fat cell volume and fat mass in ESAT is depicted in Fig. 3. In subjects who had not been subjected to weight reduction (\( n = 124 \) including cohort 1 at baseline and cohort 2), a curve-linear relationship between the two parameters was observed. In the women who were re-examined after
RYGB \( (n = 62) \), the relationship was still curve linear but the position of the curve was markedly different from the one prior to surgery \( (P = 0.03 \text{ by Student } t \text{ test}) \). The fitted fat cell volume at a particular fat mass was much smaller in the weight-reduced subjects than in the whole group of non-weight-reduced subjects. This suggests that the relationship between fat mass and fat cell volume was altered after weight reduction.

**CONCLUSIONS**

In this prospective study, we show that marked weight reduction is associated with a decrease in fat cell volume (but not number) in subcutaneous adipose tissue, which is significantly associated with improvements in insulin sensitivity. Furthermore, our present study confirms earlier observations \( (6) \) that decreased visceral, but not subcutaneous fat mass, correlated significantly with improvements in metabolic risk factors, including insulin sensitivity and plasma triglycerides. A similar lack of associations was observed for android and gynoid fat (mostly composed of subcutaneous fat) as for ESAT, further supporting the notion that a decrease in subcutaneous fat mass per se has little impact on improvements in insulin sensitivity. Unfortunately, we cannot say if a decrease in visceral fat cell volume may also be important since this measure could not, for obvious ethical reasons, be obtained after weight reduction. It is of interest to note that when the present prospective cohort was investigated at baseline, subcutaneous fat cell volume correlated more strongly than visceral fat cell volume with insulin sensitivity \( (15) \). This suggests that for subcutaneous adipose tissue, fat cell size may be of greater importance than fat mass. It is possible that the lack of effect of liposuction on the metabolic profile \( (3,4) \) is dependent on the fact that such an intervention does not alter the size of fat cells in the remaining subcutaneous adipose tissue. A decrease in subcutaneous fat cell size rather than a decrease in subcutaneous adipose mass per se may therefore be of greater importance for improvement of insulin sensitivity after weight loss. At present we cannot identify which functional aspects related to fat cell size play a causal role in the reversal of insulin resistance after weight loss. Alterations in adipocyte turnover resulting in adipose remodeling could play a role. This notion is based on the observation that when the mean curve fit for ESAT versus fat cell volume was determined, the curve for women investigated at baseline was significantly different from that obtained with women examined after RYGB. According to previously published estimates of human adipocyte turnover data, \( \sim 20\% \) of all fat cells are renewed after 2 years \( (17) \), which was the mean difference in time between the first and second examination. It could be speculated that these new fat cells, developed during at least a period of caloric restriction, may have a different size than the fat cells they replaced.

We observed some correlations between changes in fat cell size or fat mass parameters and various measures of cholesterol and blood pressure. These associations, although interesting, were not studied in detail due to possible effects of concomitant medications and the fact that the focus of this study was effects on insulin sensitivity.

Admittedly, our study has some caveats. We only studied fat cells from the subcutaneous umbilical region and we cannot extrapolate our findings to other subcutaneous fat depots. In fact, fat cell size differs slightly between abdominal and peripheral subcutaneous adipose regions \( (23,24) \), which may have an impact on the relationship between fat cell size and metabolic profile \( (23) \). We used DEXA instead of computed tomography or magnetic resonance imaging to estimate fat mass content in different adipose regions and we cannot exclude that this might have influenced the results. However, the DEXA method shows a very high concordance with computed tomography measurement also for determination of visceral fat mass \( (20) \). Moreover, we only studied women and cannot exclude the possibility that there may be sex-specific differences. Also, since treatment with pharmacological agents affecting metabolism is common in patients undergoing bariatric surgery, we cannot exclude the possibility that this could have affected our results. Finally, all women were treated with RYGB, which may also cause hormonal changes independent of weight loss \( (25) \). Although not likely, the outcome might have been different if gastric volume restriction surgery would have been used instead. Other factors not examined in this study might also contribute to metabolic improvements after weight loss. For example, nutritional factors and changes in physical activity

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Table 3—Correlation between changes in \( M \) value (dependent) and changes in subcutaneous fat cell volume and other variables tested with multiple regression analyses

<table>
<thead>
<tr>
<th>Covariable</th>
<th>Partial ( r )</th>
<th>( P ) value</th>
<th>Covariable</th>
<th>Partial ( r )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Android fat mass</td>
<td>0.32</td>
<td>0.04</td>
<td>ESAT</td>
<td>-0.003</td>
<td>0.99</td>
</tr>
<tr>
<td>Gynoid fat mass</td>
<td>0.40</td>
<td>0.01</td>
<td>Android fat mass</td>
<td>-0.05</td>
<td>0.79</td>
</tr>
<tr>
<td>Android fat/gynoid fat ratio</td>
<td>0.36</td>
<td>0.04</td>
<td>Gynoid fat mass</td>
<td>-0.10</td>
<td>0.53</td>
</tr>
<tr>
<td>EVAT</td>
<td>0.28</td>
<td>0.07</td>
<td>Android fat/gynoid fat ratio</td>
<td>-0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>0.33</td>
<td>0.07</td>
<td>EVAT</td>
<td>0.09</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Figure 3—Relationship between mean fat cell volume and ESAT. Black line is the relationship for all subjects at a baseline non-weight-reduced state. Gray line is the relationship in subjects investigated after weight reduction. The curve-linear relationships between baseline and the weight-reduced state were significantly different \( (P = 0.03 \text{ with Student } t \text{ test}) \).
may have metabolic effects that are not related to adipose tissue when body weight is reduced by lifestyle factors (2,26–28).

Measuring fat cell size might have important implications. Degree of obesity is usually just defined by the amount and (sometimes) distribution of body fat. As recently discussed, other parameters are needed to better classify subtypes of obesity that have different impacts on the risk of developing type 2 diabetes and other obesity complications (29,30). One such parameter could be subcutaneous fat cell size, which is a measure of quality, rather than quantity, of fat. It is easy to obtain small subcutaneous fat biopsies, and the size of the fat cells can readily be determined histologically (10,12).

In conclusion, a decrease in subcutaneous fat cell volume rather than a decrease in subcutaneous adipose tissue mass is associated with improvements in insulin sensitivity after marked weight loss. An altered relationship between fat mass and fat cell volume (i.e., remodeling) may at least in part explain the findings.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. D.P.A. and M.R. designed the study, recruited and examined the obese women, and wrote the first version of the manuscript. D.E.H., Å.Tho., E.T., V.Q., E.N., A.Tho., M.W., P.L., J.H., I.D., and N.M. recruited and examined the obese women. E.A. and P.A. designed the study and wrote the first version of the manuscript. P.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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