Body composition determines direct FFA storage pattern in overweight women

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OBESE HUMANS WITH A PREFERENTIAL ACCUMULATION of fat in the abdominal region are characterized more frequently by insulin resistance, type 2 diabetes, dyslipidemia, and elevated plasma free fatty acids (FFA) (20, 27) compared with those with lower body fat distribution (11). Development of these different obesity phenotypes must arise from differences in either regional lipid storage, regional lipolysis, or a combination of both. However, studies of storage and lipolysis have so far not provided clear evidence toward an explanation for the differences in body fat distribution (25).

Studies of fatty acid storage have investigated primarily meal fatty acid storage using radioactive or stable fatty acid tracers, as first described by Björntorp et al. (3). This allows for quantification of the amount of dietary fatty acids stored in different regional adipose tissues. Meal fat storage in upper body subcutaneous (UBSQ) fat has been reported to be greater (16) or similar (23, 26) when compared with lower body subcutaneous (LBSQ) fat storage in women. Moreover, in response to a high-fat meal, women showed a more efficient storage into LBSQ fat compared with men (23).

Previously, direct storage of FFA into adipose tissue has been considered to occur only in the postprandial state (2, 4). Recently, however, Shadid et al. (22) demonstrated that direct storage of circulating FFA into subcutaneous adipose tissue is also quantitatively significant in the postabsorptive state. This hitherto less explored pathway accounted for the disposal of ~8% of FFA into subcutaneous adipose tissue in women (22), and FFA storage has been reported to be greater in LBSQ fat compared with UBSQ fat in women (13). In addition, in women, direct FFA storage per adipocyte increased as a function of fat mass (FM) in subcutaneous leg fat, whereas this was not the case in either UBSQ fat or visceral fat (14). This could indicate that the direct FFA storage pathway might play a role in favoring lower body fat accumulation in some women. However, it still remains unknown how established phenotypic differences in body fat distribution affect direct FFA storage. Therefore, the aim of the present study was to investigate the impact of upper body and lower body obesity phenotypes on direct FFA storage. In addition, we wanted to compare these storage patterns with that of lean women. Our hypothesis was that in upper body overweight/obese (UBO) women, greater direct FFA storage occurs in UBSQ subcutaneous fat compared with LBSQ fat, whereas in lower body overweight/obese (LBO) women, greater direct FFA storage occurs in LBSQ fat.

METHODS

Subjects. The study protocol was approved by the local Ethics Committee (Central Denmark Region Committees on Biomedical Research Ethics), and written, informed consent was obtained from all participants.

Twenty-eight healthy, premenopausal women were recruited so that 10 were UBO [waist/hip ratio (WHR) >0.85, BMI >28 kg/m²), 11 were LBO (WHR <0.80, BMI >28 kg/m²), and seven were lean (BMI <25 kg/m²). They participated in a study of VLDL-triglyceride (TG) metabolism in women with different obesity phenotypes. Data on VLDL-TG kinetics (8) and VLDL-TG storage in adipose tissue (19) have been published previously. All women were studied in the luteal phase. The two groups of overweight/obese women were matched according to BMI to ensure comparability. All participants were normotensive, were nonsmokers, used no medication except oral contraceptives, and had a normal blood count and chemistry panel documented before participation.

Protocol. The study protocol has been described in detail previously (8). In brief, 1 wk prior to the study day volunteers had a dual-energy X-ray absorptiometry (DEXA) scan and an abdominal CT scan at the L2–L3 interspace to determine body fat mass and composition. The last 3 days before the study day, participants were provided all of their meals from the hospital kitchen to ensure...
individual isocaloric diets with a fixed macronutrient intake (55% carbohydrate, 30% lipid, and 15% protein). All participants were admitted to the research unit the evening before and spent the night there. All were studied after a 10-h overnight fast. At baseline a bolus of [1-14C]triolein VLDL tracer was administered intravenously, followed 4 h later by an intravenous bolus of [9,10-3H]palmitate. Thirty minutes after the palmitate bolus, UBSQ (lateral to the umbilicus) and LBSQ (inner thigh) fat biopsies were collected. The biopsies were rinsed immediately with ice-cold saline and the lipids extracted, and the specific activity (SA; dpm/g lipid) was calculated as described previously (16). In brief, extracted lipid was weighed, scintillation cocktail (10 ml of Optiphase HiSafe 2; Wallac) was added and mixed thoroughly, and 3H activity (dpm) was measured using a liquid scintillation counter (Wallac 1800; Beckman) to <2% counting error.

Body composition. Total body FM, leg fat, fat percent, and fat-free mass (FFM) were measured by DEXA (QDR-2000) 1 wk prior to the study day. Upper body and visceral FM were assessed using the CT measurements of intra-abdominal and subcutaneous adipose tissue combined with FM measured by DEXA, as described previously (12). Upper body subcutaneous FM was estimated as the difference between upper body fat (DEXA) and visceral fat.

Tracer preparation. Labeling of VLDL-TG with [1-14C]triolein (14C at carbon 1 of FAs) was performed as described previously (7). In short, a fasting, 80-ml venous blood sample was obtained aseptically from the participant. The plasma was transferred to a sterile glass tube containing 40 μCi [1-14C]triolein and sonicated at 37°C for 6 h. Hereafter, the VLDL fraction was separated from plasma by ultracentrifugation (40,000 rpm for 18 h at 10°C, using a sterile 1.006 g/cm³ saline solution). The ex vivo 14C-labeled VLDL-TG sample was kept at 5°C until the examination day, where it was reinfused into the participant. Sterility of the VLDL-TG solution was ensured by the participant. Sterility of the VLDL-TG solution was ensured by the previously (16). In brief, extracted lipid was weighed, scintillation cocktail (10 ml of Optiphase HiSafe 2; Wallac) was added and mixed thoroughly, and 3H activity (dpm) was measured using a liquid scintillation counter (Wallac 1800; Beckman) to <2% counting error.

Adipose tissue FFA storage and fat cell size. Palmitate was chosen as a representative fatty acid to allow assessment of FFA kinetics because its metabolic properties are largely representative of the remaining FFA (10). Previous studies have demonstrated only 3% because its metabolic properties are largely representative of the otherwise produced VLDL-TG particles (7).

Fatty acid patterns in VLDL-TG particles that are indistinguishable from endogenous produced VLDL-TG particles (7).

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RESULTS

Characteristics of the three study groups are presented in Table 1. By design, both groups of overweight women had comparable BMI and a significantly greater FM than lean women. Fat cell size of UBSQ fat was significantly greater in UBO women compared with lean women. Fat cell size in LBSQ fat was significantly greater in both groups of overweight women compared with the lean women. No significant difference was observed in plasma FFA concentration between the three groups.

Palmitate storage. Palmitate storage data are summarized in Table 2. Overall, fractional FFA storage was greater in LBSQ fat compared with UBSQ fat in the LBO (P < 0.01) and the lean (P = 0.03) groups (Fig. 1). FFA SA (dpm/g) in UBSQ compared with LBSQ fat was not significantly different in any of the three groups (Fig. 2). In addition, both UBO and lean women had comparable FFA storage per 10⁶ fat cells in UBSQ and LBSQ

| Table 1. Basal characteristics of the UBO, LBO, and lean groups |
|-------------------|-------------------|-------------------|-------------------|
| UBO (n = 10)      | LBO (n = 11)      | Lean (n = 7)      | P Value          |
| Age, yr           | 42 (27–51)        | 37 (26–53)        | 41 (20–51)       | 0.59             |
| Weight, kg        | 91.4 ± 10.0*      | 86.3 ± 6.6*       | 66.0 ± 6.3       | <0.001           |
| BMI, kg/m²        | 32.3 ± 2.3*       | 30.5 ± 1.8*       | 23.2 ± 1.6       | <0.001           |
| Waist/hip ratio   | 0.91 ± 0.05       | 0.77 ± 0.04†      | 0.80 ± 0.07†     | <0.001           |
| Total mass fat, kg| 36.4 ± 4.2        | 35.6 ± 3.7*       | 20.8 ± 4.2       | <0.001           |
| UBSQ, kg          | 13.5 ± 2.6*       | 11.7 ± 2.3*       | 5.9 ± 1.8        | <0.001           |
| LBSQ, kg          | 12.9 ± 1.6*       | 14.8 ± 1.9*       | 9.2 ± 2.1        | <0.001           |
| Visceral fat, kg  | 4.8 ± 1.5*        | 4.2 ± 1.6         | 2.5 ± 1.0        | 0.01             |
| Abdominal fat cell size, pl | 628 ± 273*      | 472 ± 103         | 369 ± 145        | 0.04             |
| Femoral fat cell size, pl | 591 ± 154*      | 618 ± 140*        | 337 ± 80         | <0.001           |
| FFA, mmol/l       | 0.55 ± 0.11       | 0.52 ± 0.07       | 0.50 ± 0.08      | 0.56             |

All values are means ± SD or median (range). UBO, upper body overweight; LBO, lower body overweight; UBSQ, upper body subcutaneous; LBSQ, lower body subcutaneous. *P < 0.05 compared with lean group; †P < 0.05 compared with UBO group.
Table 2. FFA storage of the UBO, LBO, and lean groups

<table>
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<th>UBO</th>
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<tr>
<td>UBSQ fat</td>
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<tr>
<td>Fractional FFA storage</td>
<td>0.06 (0.03–0.08)*</td>
<td>0.04 (0.03–0.07)</td>
<td>0.03 (0.01–0.04)</td>
<td>*P &lt; 0.01</td>
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<tr>
<td>SA, dpm/g lipid</td>
<td>334 (259–610)</td>
<td>326 (236–477)*</td>
<td>402 (331–558)</td>
<td>*P &lt; 0.05</td>
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<td>Storage, dpm/min−1·10⁶ fat cells</td>
<td>221 (88–346)</td>
<td>135 (103–215)</td>
<td>152 (54–248)</td>
<td>NS</td>
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<tr>
<td>LBSQ fat</td>
<td></td>
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<tr>
<td>Fractional FFA storage</td>
<td>0.06 (0.04–0.09)</td>
<td>0.06 (0.04–0.10)†</td>
<td>0.05 (0.04–0.07)†</td>
<td>NS</td>
</tr>
<tr>
<td>SA, dpm/g lipid</td>
<td>391 (243–701)</td>
<td>320 (248–573)</td>
<td>455 (374–566)</td>
<td>*P &lt; 0.09</td>
</tr>
<tr>
<td>Storage, dpm/10⁶ fat cells</td>
<td>174 (125–575)</td>
<td>171 (129–412)†</td>
<td>123 (88–209)</td>
<td>*P &lt; 0.05</td>
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All values are median (range). FFA, free fatty acids; SA, specific activity; NS, not significant. *P < 0.05 compared with lean group; †P < 0.05 compared with UBSQ fat.

Fat. However, LBO women displayed significantly greater storage per 10⁶ fat cells in LBSQ compared with UBSQ fat (P < 0.01; Fig. 3).

In accord with the differences in UBSQ FM, fractional FFA storage in UBSQ fat was significantly greater in UBO women compared with lean women, whereas no significant difference was found between UBO and LBO women or between LBO and lean women [UBO: 0.06 (0.03–0.08); LBO: 0.04 (0.03–0.07); lean: 0.03 (0.01–0.04); ANOVA, P < 0.01; Fig. 1A]. In contrast, despite differences in LBSQ FM, the fractional FFA storage in LBSQ fat was similar in all groups [UBO: 0.06 (0.04–0.09); LBO: 0.06 (0.04–0.10); lean: 0.05 (0.04–0.07); ANOVA, not significant (NS); Fig. 1B].

The FFA SA in UBSQ fat was significantly different between the three groups [UBO: 334 (259–610) dpm/g lipid; LBO: 326 (236–477) dpm/g lipid; lean: 455 (374–566) dpm/g lipid; ANOVA, P < 0.05], with significantly greater storage in lean women compared with UBO women (P < 0.05; Fig. 2A). In LBSQ fat a similar tendency was seen, although it was not statistically significant [UBO: 391 (243–701) dpm/g lipid; LBO: 320 (248–573) dpm/g lipid; lean: 455 (374–566) dpm/g lipid; ANOVA, P = 0.09; Fig. 2B]. With respect to FFA storage per 10⁶ fat cells in UBSQ fat, no significant differences were observed between the three groups [UBO: 221 (88–346) dpm/10⁶ cells; LBO: 135 (103–215) dpm/10⁶ cells; lean: 152 (54–248) dpm/10⁶ cells; ANOVA, NS; Fig. 3A]. However, in LBSQ fat, FFA storage per 10⁶ fat cells was significantly different between the three groups [UBO: 174 (125–575) dpm/10⁶ cells; LBO: 171 (129–412) dpm/10⁶ cells; lean: 123 (88–209) dpm/10⁶ cells; ANOVA, P < 0.05]. However, the post hoc analysis failed to detect significant differences between the individual groups (Fig. 3B).

The subjects in the two obese groups did not differ significantly in visceral fat mass; therefore, an effect of UBO may have been overlooked. To test whether visceral obesity predicted FFA storage, our obese subjects were regrouped according to visceral fat mass (above vs. below median), as done previously (6). The reclassification of our subjects changed the status of four subjects but did not change the overall picture of our findings.

Palmitate storage and plasma FFA concentration. The relationship between plasma FFA concentration and fractional FFA storage was assessed by linear regression analysis, with adjustment for group. The fractional FFA storage in UBSQ fat was not significantly associated with plasma FFA concentration (r² = 0.10, NS; Fig. 4A). However, in LBSQ fat, plasma FFA concentration significantly predicted fractional FFA storage [r² = 0.25 (P < 0.01); Fig. 4B]. No significant group effect was observed.

Palmitate storage and regional FM. To assess the relationship between FM and FFA storage, linear regression analysis with adjustment for group was performed. No significant group effect was observed with respect to FFA SA or storage per 10⁶ fat cells. Neither FFA SA nor FFA storage per 10⁶ fat cells was significantly predicted by regional FM in the UBSQ or LBSQ fat (Fig. 5, A–D).

Palmitate storage vs. VLDL-TG storage. Previously, we reported data from the subjects in the present study regarding VLDL-TG storage in UBSQ and LBSQ fat (19). Using linear regression analysis with fractional FFA storage as the dependent variable and fractional VLDL-TG fatty acid storage as the independent variable and adjustment for group, we found a

Fig. 1. Fraction of bolus of free fatty acids (FFA) taken up in upper body subcutaneous (UBSQ) and lower body subcutaneous (LBSQ) adipose tissues. A: fractional UBSQ FFA storage. B: fractional LBSQ FFA storage. Boxes signify quartiles and the median, with whiskers showing range. *P < 0.01 compared with fractional UBSQ storage; *P < 0.05 compared with fractional UBSQ storage. UBO, upper body overweight; LBO, lower body overweight; NS, not significant.

Fig. 2. FFA storage per gram of lipid in UBSQ and LBSQ adipose tissues. A: UBSQ FFA tracer storage (dpm/g lipid). B: LBSQ FFA tracer storage (dpm/g lipid). Boxes signify quartiles and the median, with whiskers showing range.
significant relationship between VLDL-TG fatty acid and FFA fractional storage in UBSQ fat (\(P < 0.01\)). A steeper slope was observed in the UBO group compared with both the LBO (\(P = 0.03\)) and the lean (\(P = 0.01\)) group (Fig. 6A). Similarly, in LBSQ fat, VLDL-TG fatty acid and FFA fractional storage were significantly related when adjusted for group (\(P = 0.03\)). No significant between-group effect was observed (Fig. 6B).

**DISCUSSION**

This is the first study to measure direct plasma FFA and VLDL-TG fatty acid storage in abdominal and femoral subcutaneous adipose tissue in groups of lean and overweight women with well-defined obesity phenotypes. In contrast to previous studies, we recruited our subjects in three well-defined groups, which enabled us to study the effect of UBO and LBO on direct FFA storage, and we report several novel findings. First, in LBO and lean women, but not UBO women, fractional FFA storage is greater in the LBSQ fat depot compared with the UBSQ fat depot. In the LBO women, this difference can be explained by a significantly greater storage per fat cell number in the LBSQ fat depot, not by different storage per gram of fat. Second, fractional FFA storage is greater in the UBSQ fat depot in UBO women compared with lean women, whereas there is no significant difference in LBSQ fat storage between UBO, LBO, and lean women. Finally, direct plasma FFA storage is significantly associated with the VLDL-TG fatty acid storage, indicating a common rate-limiting step [not lipoprotein lipase (LPL)] of adipose tissue storage for these two fatty acid sources.

Adipose acid storage can be presented in several different ways. We have chosen to report direct fatty acid storage in the entire regional depot, i.e., as fractional FFA storage, as well as FFA storage per gram lipid, and per \(10^6\) fat cells in the present study. Expressing storage in an entire depot provides information regarding trafficking of fatty acids into the particular regional depots. However, expressing storage per gram lipid allows comparison of the storage efficiency of one depot with another and thus reflects the metabolic task of the adipose tissue. Finally, storage expressed per number of fat cells provides information regarding adipocyte function on a cellular level.

A novel finding of this study is the greater direct fractional FFA storage in the UBSQ fat depot of UBO women compared with lean women. However, we were unable to demonstrate any association between the UBSQ FM and the FFA storage per gram lipid or per \(10^6\) fat cells. However, this is in accord with previous reports of lower (14) or unchanged (13) storage efficiency in relation to increasing UBSQ FM and indicates that direct FFA uptake is not downregulated despite increased FM, thereby leading to the observed greater UBSQ storage in UBO women. In contrast, meal fatty acid storage has been reported to be downregulated in obesity (18).

Specific activity per gram fat can be used to compare how adipose tissue depots compete for circulating FFA despite differences in body sizes. This is due to the fact that the fraction of tracer taken up is equal to the fraction of tracee FFA present in plasma at the time of tracer infusion. In addition, the important question of “dilution” into a greater adipose tissue bed can be analyzed by correlating the FFA SA in fat with the FM of a given depot (leg or abdominal). The typical dilution pattern would present as an inverse relationship, whereas an increased or unaltered efficiency would present as a positive or constant relationship. Although the storage per gram of lipid was similar in UBSQ and LBSQ fat in each of the three groups, LBO women had significantly greater storage per \(10^6\) fat cells in LBSQ fat compared with UBSQ fat. This suggests that LBSQ adipocytes in LBO women are more active with respect to FFA storage, which may be a contributory factor for their development of the LBO phenotype. The finding of similar FFA SA in UBSQ and LBSQ fat in the present study is in agreement with the results of two previous studies of direct FFA storage (14, 22). However, in another recent study, Koutsari et al. (13) reported greater storage, both per gram fat and per \(10^6\) fat cells, in LBSQ fat compared with UBSQ fat in women with a wide range of body composition and adiposity. The latter study examined a much larger group of women compared with prior studies (53 vs. 12 women) and therefore may have been able to detect smaller differences between UBSQ and LBSQ fat storage. The present study, however, differs from the above-mentioned studies in that we compared groups of well-characterized obesity phenotypes, with the focus on the impact of differences in body fat distribution. Furthermore, we found that the FFA SA in UBSQ adipose tissue was lower in UBO and LBO women compared with lean women; however, it was significant only in the LBO group. These results agree with previous studies suggesting that lower storage per gram of lipid in overweight women can be interpreted as simple dilution of tracer in a larger fat depot (14). However, we did not observe a negative correlation between SA and FM, as would be expected in the case of simple
dilution. This could indicate a downregulation of FFA storage in a progressively larger depot. The potential mechanisms for a downregulation may involve altered acyl-CoA synthetase and CD36 activity, as suggested recently (1). Unfortunately, we were not able to measure these potential mechanisms in the present study.

Fractional FFA storage in LBSQ was significantly associated with plasma FFA concentration. A similar, albeit not statistically significant, pattern was observed for UBSQ fat. This finding is consistent with recent data by Koutsari et al. (13), who reported a significant positive relationship between direct FFA storage and plasma FFA concentration. Our data suggest that the efficiency of direct FFA storage further increases in people with greater FFA concentrations. Although increased enzyme activity of lipogenic enzymes in subcutaneous fat is positively related to the amount of direct FFA storage (1, 13), the extent to which passive or active storage processes such as adipocyte lipogenic enzymes participate in the proportional increase in direct storage remains to be elucidated.

Interestingly, we were able to demonstrate a significant positive correlation between FFA and VLDL-TG-associated fatty acid storage in both UBSQ and LBSQ fat. This novel finding indicates a common pathway for lipid storage in adipose tissue. Thus, once fatty acids are released from VLDL-TG particles through the action of endothelium-associated LPL, they may be subject to the same rate-limiting storage steps in adipose tissue as plasma FFA. Therefore, it seems that LPL activity is not the rate-limiting step in the postabsorptive state. This is in accord with previous findings from our group showing that variations in adipose tissue LPL activity activity did not determine rates of VLDL-TG storage in a direct quantitative manner (19). These findings suggest that other factors rather than LPL activity regulate the VLDL-TG tissue storage, and LPL may be present in excess of what is needed to regulate VLDL-TG uptake. However, we studied fasting subjects and cannot exclude that LPL activity is perhaps rate limiting after a high-fat diet where increased chylomicron concentrations are present (23). Further studies are needed to determine the impact of LPL in low- and high-fat situations.

Some limitations should be acknowledged. First, a portion of the FFA tracer could have entered the adipose tissue via VLDL-TG; however, the slow turnover of VLDL-TG compared with FFA makes this unlikely after only 30 min (21). Furthermore, only ~4% of the tracer traversed VLDL-TG over 9.5 h in a previous study of direct FFA storage (14). Second, our sample size may be too small. Thus, type 2 errors cannot be excluded. Third, although clearly separated by WHR, both UBO and LBO women were well matched for BMI, resulting in no statistically significant differences in UBSQ or LBSQ fat mass. This may mask potential differences between the two phenotypes. Fourth, despite previous studies having demonstrated direct FFA storage in visceral fat as well as in subcutaneous fat (9, 14), we were not able to obtain biopsies from visceral fat due to the inaccessible anatomic location. Therefore, we cannot extend our findings to the visceral fat depot.

In conclusion, we report the impact of body fat distribution on direct FFA storage. LBO women take up FFA more efficiently in LBSQ fat cells, which may play a role in development of LBO. In addition, we report differences in direct FFA storage between different obesity phenotypes. Finally, we found that the storage of FFA is associated with the VLDL-TG storage, indicating a common pathway for lipid storage in adipose tissue.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
AUTHOR CONTRIBUTIONS

E.S. analyzed the data; E.S., M.J., and S.N. interpreted the results of the experiments; E.S. prepared the figures; E.S. drafted the manuscript; E.S., L.C.G., B.N., M.J., and S.N. approved the final version of the manuscript; L.C.G. and B.N. performed the experiments; L.C.G., B.N., M.J., and S.N. edited and revised the manuscript; S.N. did the conception and design of the research.

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