Similar VLDL-TG Storage in Visceral and Subcutaneous Fat in Obese and Lean Women

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OBJECTIVE—Excess visceral fat accumulation is associated with the metabolic disturbances of obesity. Differential lipid redistribution through lipoproteins may affect body fat distribution. This is the first study to investigate VLDL-triglyceride (VLDL-TG) storage in visceral fat.

RESEARCH DESIGN AND METHODS—Nine upper-body obese (UBO; waist circumference >88 cm) and six lean (waist circumference <80 cm) women scheduled for elective tubal ligation surgery were studied. VLDL-TG storage in visceral, upper-body subcutaneous (UBSQ), and lower-body subcutaneous (LBSQ) fat were measured with [9,10-^{3}H]-triolein–labeled VLDL.

RESULTS—VLDL-TG storage in visceral fat accounted for only ~0.8% of VLDL-TG turnover in UBO and lean women, respectively. A significantly larger proportion of VLDL-TG turnover was stored in UBSQ (~5%) and LBSQ (~4%) fat. The VLDL-TG fractional storage was similar in UBO and lean women for all regional depots. VLDL-TG fractional storage and VLDL-TG concentration were correlated in UBO women in UBSQ fat (r = 0.68, P = 0.04), whereas an inverse association was observed for lean women in visceral (r = −0.89, P = 0.02) and LBSQ (r = −0.87, P = 0.02) fat.

CONCLUSIONS—VLDL-TG storage efficiency is similar in all regional fat depots, and trafficking of VLDL-TG into different adipose tissue depots is similar in UBO and lean women. Postabsorptive VLDL-TG storage is unlikely to be of major importance in the development of preferential upper-body fat distribution in obese women. ***Diabetes*** **60:2787–2791, 2011**

Upper-body obesity, especially when associated with visceral fat accumulation, is related to the development of metabolic abnormalities, such as insulin resistance, type 2 diabetes, and dyslipidemia (1,2). In contrast, lower-body obesity does not exhibit these abnormalities (3,4). The mechanism behind the development of these different obesity phenotypes remains unclear (5,6). Studies have not provided clear evidence to suggest that differences in regional lipolysis promote the development of differences in adipose tissue distribution (6–8). Moreover, studies of meal fat storage and direct plasma free fatty acid (FFA) storage have failed to demonstrate definite differences, with reports showing greater (9,10) and similar (6,11) storage in visceral compared with subcutaneous adipose tissue in lean and obese men and women.

Differences between chylomicron and VLDL-triglyceride (VLDL-TG) uptake in different regional adipose tissues (12) underscore that studies of VLDL-TG storage are warranted. By using an ex vivo–labeled VLDL-TG tracer, we recently reported that VLDL-TG adipose tissue fatty acid storage was similar in upper-body subcutaneous (UBSQ) and lower-body subcutaneous (LBSQ) fat in lean and obese women (13) and in obese and type 2 diabetic men (14). Thus far, no studies have investigated VLDL-TG fatty acid storage in visceral adipose tissue.

The aim of this study was to test the hypothesis that VLDL-TG fatty acid storage is greater in visceral adipose tissue compared with LBSQ and UBSQ adipose tissue. We wanted to test this hypothesis in both upper-body obese (UBO) and lean women. A secondary aim was to assess whether the storage pattern differed between UBO and lean women.

RESEARCH DESIGN AND METHODS

The protocol was approved by the local ethics committee, and informed consent was obtained from all participants. Nine UBO women (waist circumference >88 cm) and 6 lean women (waist circumference <80 cm) scheduled for voluntary laparoscopic tubal occlusion were recruited. All were nonsmokers, used no medication except oral contraceptives, and had a normal blood and chemistry panel. One week before the study day, a blood sample for VLDL-TG tracer preparation was obtained after a 10–14 h overnight fast. Dual X-ray absorptiometry scan and abdominal computed tomography scan at the L_2-L_3 interspace were performed to obtain anthropometric indices (15).

Protocol. Figure 1 illustrates the study protocol. Participants were admitted to the Elective Surgery Section on the day of the procedure at 0745 h (t = −15 min) after a 10–14 h overnight fast. A catheter was placed in an antecubital vein, and at 0800 h (t = 0 min) a bolus of [9,10-^{3}H]VLDL-TG was infused. At t = 165 min, the participant was taken to the operating room and anesthetized. A biopsy from omental fat was obtained immediately after the tubal occlusion procedure (t = ~190 min). Immediately after the laparoscopic procedure, biopsies were obtained from UBSQ and LBSQ adipose tissue (t = ~200 min). After blood sampling at 210 min, the participants were awakened from anesthesia and followed a normal postoperative procedure.

VLDL-TG tracer preparation. A 60-mL blood sample was obtained under sterile conditions from each volunteer. Plasma VLDL-TG was labeled with 120 μCi of [9,10-^{3}H]triolein (Perkin Elmer, Inc., Waltham, MA) as previously described (16).

Plasma VLDL-TG concentration and specific activity. VLDL-TG concentration and specific activity (SA) were measured after ultracentrifugation as previously described (16).

Adipose tissue biopsies. Omental adipose tissue biopsies were obtained from the middle of the lower edge of the omentum. The UBSQ and LBSQ adipose tissue biopsies were obtained using a liposuction technique from the periumbilical and anterior femoral regions.

Adipose tissue ^{3}H-TG SA and fat cell size. Adipose tissue lipid SA (dpm/g) was measured after lipid extraction from adipose tissue biopsies, as described previously (17). Fat cell size was determined immediately after the biopsy procedure, as described previously (18).

Calculations. VLDL-TG secretion rate was calculated using a bi-exponential fit to the plasma tracer decay curve with correction (19). VLDL-TG FA storage was calculated both as a timed storage rate (μmol/min) and as the net fraction stored over the 210 min in the entire depot, per kilogram lipid, and per 10^6 fat

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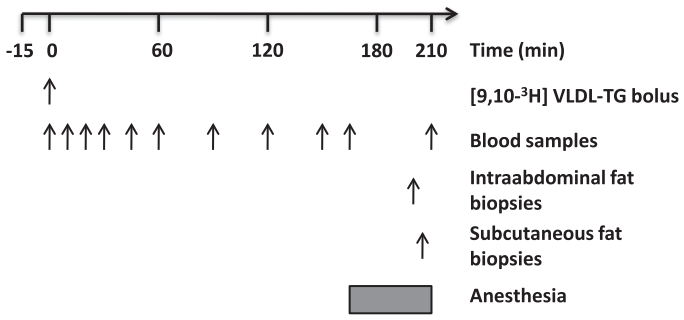

FIG. 1. Study protocol.

cells. The VLDL-TG storage rate into adipose tissue (μmol/min) was calculated as the fractional tracer storage × VLDL secretion rate.

Statistics. Data are presented as mean ± SD or median (range) as appropriate. Differences between groups were evaluated using the Student *t* test or Mann–Whitney test. Analyses of differences in regional storage were performed using repeated-measurement ANOVA with post hoc comparison using the Student *t* test for paired samples. Correlations are Pearson *r*. Statistical significance was set at $P = 0.05$.

RESULTS

Baseline data are summarized in Table 1.

VLDL-TG turnover. VLDL-TG secretion rates (52 ± 12 vs. 46 ± 11 μmol/min; not significant) and VLDL clearance rates (43 ± 13 vs. 50 ± 15 mL/min) were similar in UBO and lean women. At 210 min, the residual amount of [9,10-^{3}H]VLDL-TG in plasma was low in both groups (3.6 ± 2.0 vs. 2.2 ± 0.5%; not significant).

VLDL-TG storage. To explore the possibility of adipose tissue sample contamination by residual activity from plasma (20), residual plasma activity was plotted against activity in adipose tissue samples (dpm/g). No sign of a relationship was detected in any of the regional depots. VLDL-TG fractional storage was equivalent in UBO and lean women in each of the regional depots (visceral fat: 0.76 ± 0.48 vs. 0.81 ± 0.60%, not significant; UBSQ fat: 4.4 ± 2.9 vs. 5.7 ± 4.7%, not significant; LBSQ fat: 4.8 ± 2.8 vs. 3.9 ± 1.0%, not significant) (Fig. 2*A*). VLDL-TG storage rates in each of the different regional fat depots were also equivalent in UBO and lean women (visceral fat: 0.42 ± 0.29 vs. 0.35 ± 0.23 μmol/min, not significant; UBSQ fat: 2.3 ± 1.5 vs. 2.7 ± 2.7 μmol/min, not significant; LBSQ fat: 2.7 ± 1.9 vs. 1.8 ± 0.7 μmol/min, not significant) (Fig. 2*B*). As expected, because of lower visceral fat depot size, both fractional storage and storage rates were significantly lower in visceral fat compared with both LBSQ and UBSQ fat for both UBO and lean women (all $P < 0.05$).

The fraction of VLDL-TG storage per kilogram lipid (Fig. 2*C*) was significantly lower in UBSQ fat in UBO compared with lean women ($P = 0.04$). No significant differences were observed in the fractional VLDL-TG storage per kilogram lipid among visceral, UBSQ, and LBSQ fat depots in UBO or lean women. The fraction of VLDL-TG storage per 10^6 fat cells (Fig. 2*D*) displayed the same pattern as storage per kilogram lipid; however, no significant differences were observed between different regional adipose tissue depots or between groups.

VLDL-TG storage and regional fat mass. There was no significant correlation between fractional VLDL-TG storage per kilogram lipid and fat mass in visceral fat, UBSQ fat, or LBSQ fat. Fractional VLDL-TG storage per 10^6 fat cells was significantly associated with UBSQ fat mass in lean women ($r = 0.82$, $P < 0.05$). A similar relationship was observed in UBO women, although it was not significant ($r = 0.66$, $P = 0.08$). No significant correlations were observed with visceral or LBSQ fat.

VLDL-TG storage and VLDL-TG concentration and secretion. In lean women, fractional VLDL-TG storage in visceral ($r = -0.89$, $P = 0.02$) and LBSQ ($r = -0.87$, $P = 0.02$) fat was inversely associated with VLDL-TG concentration (Fig. 3*A*–*C*). In UBSQ fat, a similar but not significant relationship was observed (Fig. 3*B*). In UBO women, a tendency toward a positive relationship was observed between fractional VLDL-TG storage and VLDL-TG concentration in all regional fat depots, although only significant in UBSQ fat ($r = 0.68$, $P = 0.04$) (Fig. 3). No significant correlations were observed between fractional VLDL-TG storage and VLDL-TG secretion in any of the regional depots in UBO and lean women.

TABLE 1
Clinical characteristics of participants

	UBO women	Lean women	*P* value
n	9	6	
Age (years)	35 (28–40)	41 (33–43)	<0.05
Weight (kg)	82.7 ± 5.0	65.2 ± 4.9	<0.01
BMI (kg/m^2)	30.1 ± 4.0	23.5 ± 1.3	<0.01
Waist-to-hip ratio	0.86 ± 0.04	0.80 ± 0.05	<0.05
Fat free mass (kg)	51.9 ± 3.5	45.6 ± 4.2	<0.01
Total fat mass (kg)	30.8 ± 4.2	19.6 ± 3.5	<0.01
Fat (%)	37.2 ± 3.8	30.0 ± 4.6	<0.05
Visceral fat (kg)	1.9 ± 0.6	1.0 ± 0.5	<0.05
UBSC fat (kg)	14.5 ± 3.0	8.4 ± 2.3	<0.01
LBSC fat (kg)	14.4 ± 1.4	10.1 ± 1.3	<0.01
TG (mmol/L)	1.2 (0.9–1.6)	0.9 (0.8–1.2)	0.37
Total cholesterol (mmol/L)	4.8 ± 0.7	4.3 ± 0.8	0.21
LDL cholesterol (mmol/L)	2.4 ± 0.7	2.6 ± 0.6	0.71
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.9 ± 0.2	<0.01
FFA (mmol/L)	0.59 ± 0.31	0.47 ± 0.14	0.38
Insulin (pmol/L)	48.6 ± 24.6	27.6 ± 11.5	0.08
HOMA-IR	1.83 ± 0.98	0.93 ± 0.40	<0.05

Data are mean ± SD or median (range). HOMA-IR, homeostasis model assessment of insulin resistance.

DISCUSSION

This study is the first to compare direct quantitative VLDL-TG storage in visceral, UBSQ, and LBSQ fat in lean and UBO women, and the data reveal several novel results. The main findings are that visceral fat storage per kilogram lipid is equivalent to that of UBSQ and LBSQ, and therefore contributes quantitatively less to overall postabsorptive VLDL-TG storage. In addition, trafficking of VLDL-TG fatty acids for storage into visceral fat, UBSQ, and LBSQ fat depots is similar in lean and UBO women. Moreover, VLDL-TG concentration correlates positively with VLDL-TG storage in UBO women, but inversely in lean women.

Visceral adipose tissue has attracted special interest because of the anatomic location with venous drainage into the portal circulation. However, studies of visceral fat storage and lipolysis are sparse and conflicting, probably because of the obvious limitations in the ability to obtain visceral fat biopsies. In the current study, we used a newly developed tracer technique in combination with adipose tissue biopsies to measure VLDL-TG storage in visceral, UBSQ, and LBSQ adipose tissue. We hypothesized that

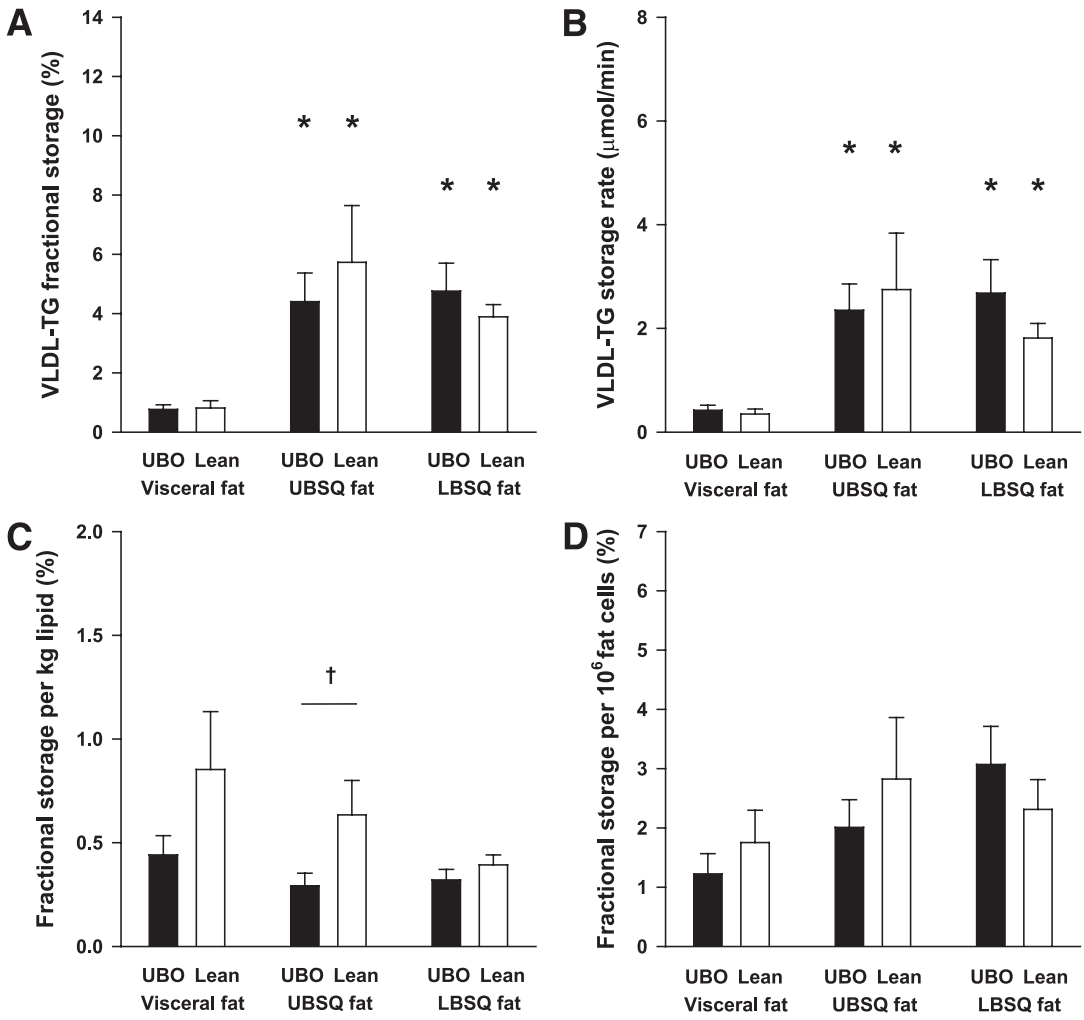

FIG. 2. *A*: VLDL-TG fractional storage in visceral, UBSQ, and LBSQ fat. *B*: VLDL-TG storage rate in visceral, UBSQ, and LBSQ fat. *C*: Fractional VLDL-TG storage per kilogram lipid in visceral, UBSQ, and LBSQ fat. *D*: Fractional VLDL-TG storage per 10^6 fat cells in visceral, UBSQ, and LBSQ fat. Error bars are SEM. *$P < 0.05$ compared with visceral fat within group. †$P < 0.05$ between UBO and lean women.

visceral fat would be more metabolically active in comparison with subcutaneous fat with a greater storage efficiency to match the potential greater lipolysis. However, we found the VLDL-TG fatty acid storage per kilogram lipid to be similar in visceral, UBSQ, and LBSQ in both UBO and lean women. Therefore, because of the smaller size of the visceral fat depot compared with the UBSQ and LBSQ depots, the relative contribution of visceral fat to the overall VLDL-TG storage is significantly lower than storage in UBSQ and LBSQ fat. This finding corroborates with studies of meal fat (11) and direct plasma FFA storage (6) in women. However, in men, two studies reported increased storage of meal fatty acids (10) and plasma FFA (9) in visceral fat compared with UBSQ fat. This suggests sex-specific differences in visceral adipose tissue biology, resulting in more avid storage in visceral adipose tissue in men compared with women, and may help explain the more distinct UBO pattern typically seen in men.

No significant differences in VLDL-TG fractional storage, storage rate, and fractional storage per kilogram lipid were observed between the individual fat depots in both UBO and lean women, indicating a similar pattern in trafficking of VLDL-TG fatty acids. We take this as evidence against VLDL-TG storage as a prominent mechanism accounting for differences in body composition. In contrast, in a recent study of UBO and lean women, we found that fractional storage of VLDL-TG in UBSQ fat was greater in UBO compared with lean women (21). Differences in selection criteria of the obese subjects (BMI and waist-to-hip ratio as opposed to waist circumference) may partly explain this difference. Moreover, UBO women with greater body weight were included in our previous study (21), and the UBO women had higher VLDL-TG secretion rates compared with lean women, whereas VLDL-TG secretion rates were similar in the current study. These differences between the two studies could indicate that differences in VLDL-TG storage are not a cause of UBO but may instead be the consequence of more pronounced metabolic disturbances of UBO.

The relationship between fractional VLDL-TG storage and VLDL-TG concentration seemed opposite in UBO and lean women. The overall picture appeared the same in all three fat depots. We are not aware of previous studies reporting on the relationship between VLDL-TG storage and VLDL-TG plasma concentrations. The opposite relationships observed in the current study suggest that in lean women the ability of adipose tissue to store VLDL-TG

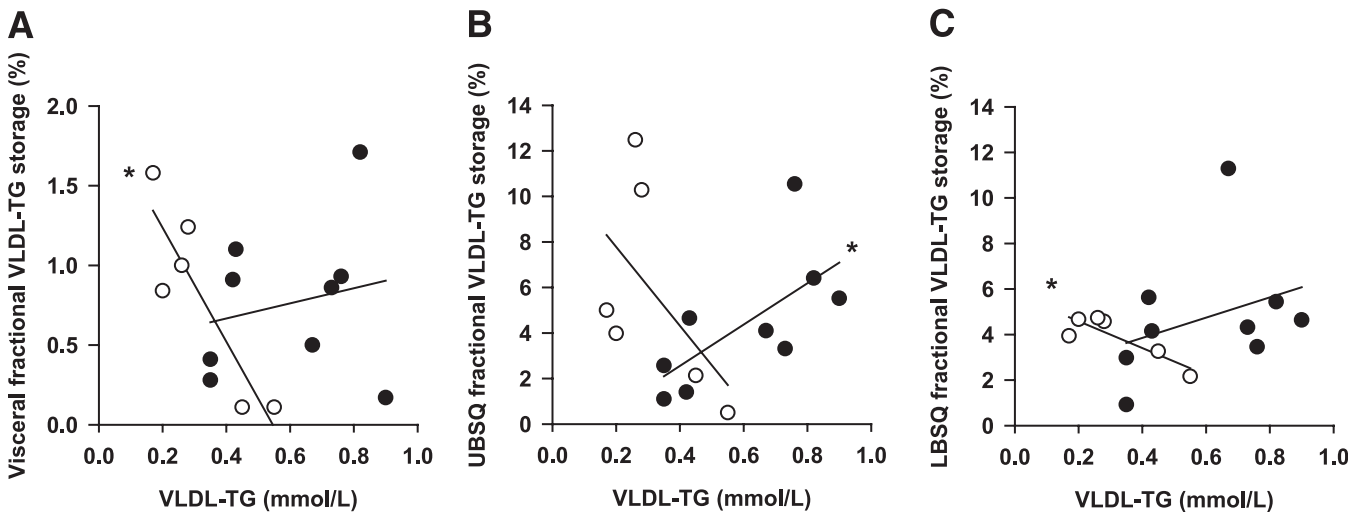

FIG. 3. Fractional VLDL-TG storage vs. VLDL-TG concentration. *A*: Visceral fat. *B*: UBSQ fat. *C*: LBSQ fat. ● = UBO, ○ = lean, *$P < 0.05$.

predicts plasma concentration, i.e., lean women with high storage capacity have low VLDL-TG and vice versa. This is not the case for UBO women, in whom the storage capacity increases with greater VLDL-TG concentration, perhaps as an indication of compensatory mechanisms that upregulate VLDL-TG storage with progressively greater VLDL-TG concentrations. This supports previous reports of VLDL-TG concentration being predicted by VLDL-TG secretion rate in UBO women (21) as opposed to VLDL-TG clearance in lean women (22).

Some limitations are acknowledged. The sample size is small, which increases the risk of type 2 error. This could be important, because even small differences may play a role in long-term lipid redistribution. Moreover, subjects were examined before and during elective surgery, i.e., during a probable stress response, which may affect lipid metabolism; however, anesthesia was not initiated until after 165 min after the bolus infusion, allowing tracer to be stored for more than 2.5 h. Finally, visceral VLDL-TG tracer storage was extrapolated from small adipose tissue biopsies sampled from the omentum. However, omental fat has been shown to be a good surrogate for visceral fat (23).

In conclusion, VLDL-TG storage per kilogram lipid is similar in visceral, UBSQ, and LBSQ fat. Furthermore, there are no significant differences between UBO and lean women in the trafficking pattern of VLDL-TG into these adipose tissue depots. In addition, VLDL-TG concentration is differently related to the storage pattern in UBO and lean women. Postabsorptive VLDL-TG storage is unlikely to be of major importance in the development of preferential upper-body fat distribution in obese women.

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E.S. designed the study, researched data, and wrote the manuscript. B.N., E.E., and M.D. researched data and reviewed the manuscript. L.P.S., L.C.G., and J.S.C. contributed to discussion and reviewed the manuscript. S.N. designed the study, researched data, contributed to discussion, and reviewed the manuscript.

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REFERENCES

1. Nielsen S, Jensen MD. Obesity and cardiovascular disease: is body structure a factor? Curr Opin Lipidol 1997;8:200–204
2. Roust LR, Jensen MD. Postprandial free fatty acid kinetics are abnormal in upper body obesity. Diabetes 1993;42:1567–1573
3. Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM. Influence of body fat distribution on free fatty acid metabolism in obesity. J Clin Invest 1989;83:1168–1173
4. Krotkiewski M, Björntorp P, Sjöström L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J Clin Invest 1983;72:1150–1162
5. Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. Splanchnic lipolysis in human obesity. J Clin Invest 2004;113:1582–1588
6. Koutsari C, Dumesic DA, Patterson BW, Votruba SB, Jensen MD. Plasma free fatty acid storage in subcutaneous and visceral adipose tissue in postabsorptive women. Diabetes 2008;57:1186–1194
7. Mårin P, Andersson B, Ottosson M, et al. The morphology and metabolism of intraabdominal adipose tissue in men. Metabolism 1992;41:1242–1248
8. Tchernof A, Bélanger C, Morisset AS, et al. Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. Diabetes 2006;55:1353–1360
9. Hannukainen JC, Kalliokoski KK, Borra RJM, et al. Higher free fatty acid uptake in visceral than in abdominal subcutaneous fat tissue in men. Obesity (Silver Spring) 2010;18:261–265
10. Mårin P, Lönn L, Andersson B, et al. Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. J Clin Endocrinol Metab 1996;81:1018–1022
11. Votruba SB, Mattison RS, Dumesic DA, Koutsari C, Jensen MD. Meal fatty acid uptake in visceral fat in women. Diabetes 2007;56:2589–2597
12. McQuaid SE, Humphreys SM, Hodson L, Fielding BA, Karpe F, Frayn KN. Femoral adipose tissue may accumulate the fat that has been recycled as VLDL and nonesterified fatty acids. Diabetes 2010;59:2465–2473
13. Nellemann B, Gormsen LC, Christiansen JS, Jensen MD, Nielsen S. Postabsorptive VLDL-TG fatty acid storage in adipose tissue in lean and obese women. Obesity (Silver Spring) 2010;18:1304–1311
14. Sørensen LP, Andersen IR, Søndergaard E, et al. Basal and insulin mediated VLDL-triglyceride kinetics in type 2 diabetic men. Diabetes 2011;60:88–96
15. Jensen MD, Kanaley JA, Reed JE, Sheedy PF. Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. Am J Clin Nutr 1995;61:274–278

16. Gormsen LC, Jensen MD, Nielsen S. Measuring VLDL-triglyceride turnover in humans using ex vivo-prepared VLDL tracer. J Lipid Res 2006;47:99–106
17. Mårin P, Rebuffé-Scrive M, Björntorp P. Uptake of triglyceride fatty acids in adipose tissue in vivo in man. Eur J Clin Invest 1990;20:158–165
18. Di Girolamo M, Mendlinger S, Fertig JW. A simple method to determine fat cell size and number in four mammalian species. Am J Physiol 1971;221:850–858
19. Sørensen LP, Gormsen LC, Nielsen S. VLDL-TG kinetics: a dual isotope study for quantifying VLDL-TG pool size, production rates, and fractional oxidation in humans. Am J Physiol Endocrinol Metab 2009;297:E1324–E1330
20. Shadid S, Koutsari C, Jensen MD. Direct free fatty acid uptake into human adipocytes in vivo: relation to body fat distribution. Diabetes 2007;56:1369–1375
21. Gormsen LC, Nellemann B, Sørensen LP, Jensen MD, Christiansen JS, Nielsen S. Impact of body composition on very-low-density lipoprotein-triglycerides kinetics. Am J Physiol Endocrinol Metab 2009;296:E165–E173
22. Mittendorfer B, Patterson BW, Klein S. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. Am J Clin Nutr 2003;77:573–579
23. Romanski SA, Nelson RM, Jensen MD. Meal fatty acid uptake in human adipose tissue: technical and experimental design issues. Am J Physiol Endocrinol Metab 2000;279:E447–E454