

Impact of body composition on very-low-density lipoprotein-triglycerides kinetics

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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* 296: E165–E173, 2009. First published November 4, 2008; doi:10.1152/ajpendo.90675.2008.—Upper body obese (UBO) subjects have greater cardiovascular disease risk than lower body obese (LBO) or lean subjects. Obesity is also associated with hypertriglyceridemia that may involve greater production and impaired removal of very-low-density lipoprotein (VLDL)-triglycerides (TG). In these studies, we assessed the impact of body composition on basal VLDL-TG production, VLDL-TG oxidation, and VLDL-TG storage. VLDL-TG kinetics were assessed in 10 UBO, 10 LBO, and 10 lean women using a bolus injection of [1-¹⁴C]VLDL-TG. VLDL-TG oxidation was measured by ¹⁴CO₂ production (hyamine trapping) and VLDL-TG adipose tissue storage by fat biopsies. Insulin sensitivity was assessed by the hyperinsulinemic-euglycemic clamp technique and body composition by dual X-ray absorptiometry in combination with computed tomography. Hepatic VLDL-TG production was significantly greater in UBO than in lean women [(μmol/min) UBO: 64.8 (SD 40.0) vs. LBO: 42.5 (SD 25.6) vs. lean: 31.8 (SD 13.3), *P* = 0.04], whereas VLDL-TG oxidation was similar in the three groups and averaged 20% of resting energy expenditure [(μmol/min) UBO: 38.3 (SD 26.5) vs. LBO: 23.5 (SD 13.5) vs. lean: 21.1 (SD 9.7), *P* = 0.09]. In UBO women, more VLDL-TG was deposited in upper body subcutaneous fat [VLDL-TG redeposition in abdominal adipose tissue (μmol/min): UBO: 5.0 (SD 2.9) vs. LBO: 4.0 (SD 3.2) vs. lean: 1.3 (SD 1.0), ANOVA *P* = 0.01]; in LBO women, more VLDL-TG was deposited in femoral fat [VLDL-TG redeposition in femoral adipose tissue (μmol/min): UBO: 5.1 (SD 3.1) vs. LBO: 5.8 (SD 4.3) vs. lean: 2.3 (SD 1.5), ANOVA *P* = 0.04]. Only a small proportion of VLDL-TG (8–16%) was partitioned into redeposition in either group. We found that elevated VLDL-TG production without concomitant increased clearance via oxidation and adipose tissue redeposition contributes to hypertriglyceridemia in UBO women.

very-low-density lipoprotein-triglycerides; body composition; obesity; tracers; fat biopsies

BODY COMPOSITION IS AN IMPORTANT PREDICTOR of obesity-related diseases, and preferential abdominal adipose tissue accumulation is more strongly associated with cardiovascular disease (CVD) and insulin resistance (9, 35, 40) than is accumulation of lower body fat (15, 33). The reason for this is not yet fully understood, but there are indications that upper body fat depots (subcutaneous and visceral) contain larger (17) and more lipolytically active (15) adipocytes, resulting in excess hepatic free fatty acid (FFA) delivery in upper body obese (UBO) individuals (29). Experimental evidence and cross-sectional

studies have demonstrated that elevated levels of FFAs affect the cardiovascular system unfavorably (30, 41) and contribute to the development of insulin resistance (36). A prominent feature of insulin resistance is hypertriglyceridemia, notably increased levels of very-low-density lipoprotein (VLDL)-triglyceride (TG).

Although lipolysis in subcutaneous adipose tissue accounts for ~75% of FFA entering the liver, excess FFA release from visceral adipocytes in UBO individuals may impact on VLDL-TG secretion (29). In the liver, FFAs are reesterified to form VLDL-TG, which is subsequently released. Studies in cell lines (6) and whole body investigations in humans (19) have demonstrated that elevated FFA levels may directly affect VLDL-TG output by the liver. Moreover, elevated FFA levels may induce hepatic insulin resistance (14), resulting in increased VLDL-TG output due to loss of the inhibitory effect of insulin (21) on VLDL-TG secretion. Thus abnormalities of regional lipolysis may contribute in a direct way to the development of hypertriglyceridemia in insulin-resistant obesity.

On the other hand, differences in regional adipose tissue triglyceride uptake may be important for the development of unfavorable adipose depots. Because a substantial proportion of FFA taken up by the liver is reesterified into VLDL-TG, knowledge of the fate of this particle may provide important information about its capacity to supply fatty acids to lean tissues for oxidation as well as to the capacity to direct fatty acids for storage in adipose tissue in different obesity phenotypes. To date, no studies have addressed the fate of VLDL- and TG-derived fatty acids, probably because of lack of robust *in vivo* methods. Regional differences in lipoprotein lipase (LPL) activity between UBO and lower body obese (LBO) women have been reported (31), which could influence regional clearance of plasma VLDL-TG into adipose tissue. VLDL-TG clearance could also be affected by the capacity of tissues that take up and oxidize VLDL-TG fatty acids. To our knowledge, the proportion of VLDL-TGs that are destined for oxidation has not been examined. If obese subjects preferentially direct VLDL-TG toward deposition instead of toward oxidation, it may provide important clues as to whether abnormalities in VLDL-TG handling contribute to the metabolic abnormalities associated with insulin-resistant obesity.

These studies were therefore designed to investigate differences in the fate of VLDL- and TG-associated fatty acids in lean and obese women with different body composition phenotypes.

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MATERIALS AND METHODS

This study was approved by the Local Ethics Committee, and each subject consented to participate in the study before the start of the screening visit.

Subjects

Twenty obese (10 UBO and 10 LBO) and 10 lean, premenopausal women were recruited through newspaper advertisements. All participants were in good health and not taking any medication except oral contraceptives. All were categorized as having a low 10-yr risk ($\leq 1\%$) of fatal CVD (4). All were studied in the luteal phase. If there was uncertainty regarding their status as premenopausal (age > 45 yr), a blood sample was taken for determination of follicle-stimulating hormone (FSH) and estradiol, and subjects were excluded if FSH > 10 IE/l and estradiol < 0.5 $\mu\text{mol/l}$. For the obese subjects, body composition inclusion criteria included waist-to-hip ratio (WHR) > 0.85 (UBO) or < 0.8 (LBO) combined with body mass index (BMI) > 28 kg/m^2 . Subjects were categorized as lean controls if their BMI < 25 kg/m^2 . All participants' lipid profiles fell within the following parameters: fasting total cholesterol < 6 mmol/l , high-density lipoprotein cholesterol > 0.9 mmol/l , low-density lipoprotein cholesterol < 5 mmol/l , TG < 2.3 mmol/l .

Homologous VLDL-TG Tracer Preparation

The ex vivo labeling procedure of VLDL-TG with $[1-^{14}\text{C}]$ triolein has been described in detail previously (10). Briefly, an 80-ml blood sample was obtained under sterile conditions from each volunteer. Plasma was separated, mixed with $[1-^{14}\text{C}]$ triolein, and subsequently sonicated in a water bath at 37°C for 6 h. The labeled plasma was then transferred to sterile Optiseal tubes (Beckman Instruments, Palo Alto, CA), covered with a saline solution ($d = 1.006$ g/ml), and centrifuged in an ultracentrifuge (Ti 50.3 rotor; Beckman Instruments) for 18 h at 40,000 rpm and 10°C . The supernatant containing the labeled VLDL fraction was finally removed with a modified Pasteur pipette, filtered, and stored under sterile conditions at 5°C . All samples were tested to ensure sterility. In addition, representative samples were tested to ensure apyrogenicity.

Protocol

Potentially eligible subjects visited the Clinical Research Laboratories after an overnight 12- to 14-h fast. Blood was obtained for determination of a lipid profile, HbA_{1c}, liver and kidney function, and complete blood count. Hip circumference was measured at the level of the trochanter major and waist circumference at the umbilical level. All WHR measurements were performed by the same investigator (Gormsen) to minimize interinvestigator variability.

Seven days before the metabolic study day, subjects who met the eligibility criteria came to the Research Laboratories after an overnight fast of 12–14 h. An 80-ml blood sample was drawn to isolate VLDL-TG for subsequent ex vivo labeling as described. At the same visit, a dual X-ray absorptiometry (DXA) scan and abdominal computed tomography (CT) scan at the L₂-L₃ interspace were performed to obtain anthropometric indexes (13). Subjects were interviewed by a dietitian who estimated their daily caloric intake. Based on the dietitian's calculations, subjects consumed a weight-maintaining diet (55% carbohydrate, 15% protein, and 30% fat) provided by the hospital kitchen during the 3 days preceding the metabolic study.

Study Day

Subjects came to the Clinical Research Laboratories at 2200 on the evening before the study and remained fasting for the remaining time of the study. At 0700, catheters were placed in an antecubital vein and a contralateral heated hand vein. The antecubital catheter was used for the bolus injection of $[1-^{14}\text{C}]$ triolein-labeled VLDL at time (t) = 0

min. The other catheter was used for drawing blood samples to determine VLDL-TG specific activity (SA) (at $t = 0, 30, 60, 120, 180, 240,$ and 300 min) and metabolite and insulin concentrations (at $t = 0, 60, 120, 180, 240, 300, 360,$ and 420 min). Breath samples were obtained every 30 min using hyamine trapping (see below) to determine $^{14}\text{CO}_2$ SA. Baseline indirect calorimetry was performed for 30 min from $t = 0$ min. From $t = 60$ min and onward, 10-min measurements were performed every hour ($t = 60, 120, 180, 240, 300, 360,$ and 420) to determine rate of CO_2 production. At $t = 270$ min, fat biopsies were obtained from the abdominal (periumbilical) and femoral (inner thigh, ~ 10 cm below the inguinal ligament) regions using a liposuction technique after local anesthesia with lidocaine. At $t = 300$ min, a 2-h constant (0.6 $\text{mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) infusion of human insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was started; based on measurements every 10 min, plasma glucose concentrations were clamped at 5.0 mmol/l by infusion of variable amounts of 20% glucose solution. Insulin sensitivity was estimated by the level of glucose infusion rate (GIR) during the hyperinsulinemic, euglycemic clamp. At 420 min, all catheters were removed, and the participants were discharged.

Body Composition

Total body fat, leg fat, fat percent, and fat-free mass were examined by DXA (QDR-2000). Upper-body and visceral fat mass were assessed using the CT measures of intra-abdominal and subcutaneous adipose tissue combined with abdominal fat mass measured by DXA as previously described (13). Upper body subcutaneous fat (UBSC) was taken as upper body fat (DXA) minus visceral fat. Leg fat was measured using the region of interest program with the DXA instrument.

Indirect Calorimetry

Resting energy expenditure and substrate oxidation rates were measured by indirect calorimetry (Deltatrac monitor; Datex Instrumentarium, Helsinki, Finland), and net lipid and glucose oxidation rates were calculated by using the nonprotein respiratory quotient from the above measurements (8).

Adipose Tissue VLDL-TG Tracer Uptake

Adipose tissue biopsies were immediately rinsed with ice-cold saline, and adipose tissue lipid SA [$\text{disintegrations}\cdot\text{min}^{-1}$ (dpm) $\cdot\text{g}$ adipose tissue lipid $^{-1}$] was measured after lipid extraction, as previously described (23). In brief, extracted lipid was weighed, scintillation cocktail (10 ml Optiphase HiSafe 2; Wallac) was added and mixed thoroughly, and ^{14}C activity (counts/min) was measured using a liquid scintillation counter (Wallac 1800; Beckman). Counts were automatically corrected for quench and converted to dpm per gram lipid.

Total regional tracer uptake was calculated as the SA multiplied by the total amount of lipid for the particular region, as assessed by the body composition measurements.

Laboratory Procedures

Plasma glucose was measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments). Serum FFA concentrations were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Serum insulin was determined by a commercial immunological kit (Dako, Glostrup, Denmark).

Breath $^{14}\text{CO}_2$ Production Rates

To measure breath $^{14}\text{CO}_2$ radioactivity, subjects exhaled through a Pasteur pipette into a solution containing 0.5 ml hyamine hydroxide in 1 M methanol (Zinsser Analytic, Berkshire, UK), 2 ml of 96% ethanol (BDH Laboratory Supplies, Poole, UK), and two drops of phenol-

phthalein. When the breath sample was passed through this trapping solution, a color change (blue to clear) occurred when exactly 0.5 mmol CO₂ was trapped in the solution. Scintillation cocktail was added and mixed thoroughly, and ¹⁴C activity was measured using liquid scintillation counting to <2% counting error. Counts were automatically corrected for quench and converted to dpm per millimole. ¹⁴CO₂ production rates were determined by multiplying breath ¹⁴CO₂ SA times CO₂ production (mmol/min) measured by indirect calorimetry.

Plasma VLDL-TG Concentration and SA

VLDL was isolated from plasma by ultracentrifugation. Approximately 3 ml of each plasma sample were transferred to Optiseal tubes, covered with a saline solution (*d* = 1.006 g/ml), and centrifuged for 18 h at 40,000 rpm and 10°C as described above. The top layer, containing VLDL, was obtained by slicing the tube ~1 cm from the top using a tube slicer (Beckman Instruments), and the exact volume was recorded. A small proportion was analyzed for TG content, and the plasma concentration of VLDL-TG was calculated. The remaining VLDL-TG was transferred to a scintillation glass vial, 10 ml of scintillation liquid was added, and the sample was measured for ¹⁴C activity using liquid scintillation counting to <2% counting error.

Calculations

VLDL-TG production. VLDL-TG and TG concentrations remained constant throughout the basal 5-h sampling period (Fig. 1). The postbolus VLDL-TG SA vs. time data from each subject was analyzed by nonlinear least-squares regression analysis using a monoexponential and a biexponential decay model. Curve fitting analyses were done by means of the computer program SigmaPlot version 9.00 for Windows (Systat Software, San Jose, CA). The preferred model was chosen on the basis of visual inspection of the VLDL-TG SA vs. time data and the adjusted *r*² (see Fig. 2). Using this approach, a biexponential equation yielded a better fit than the monoexponential ap-

proach, as assessed by the adjusted *r*². VLDL-TG SA at any time point can then be described by the following:

$$SA(t) = A_1 \times e^{(-a \times t)} + A_2 \times e^{(-b \times t)}$$

where *a* and *b* are decay constants of the early and late phase, respectively, and *A*₁ and *A*₂ denote theoretical intercept constants of the early and late slopes.

VLDL-TG production and clearance rates were then calculated as

$$\text{VLDL-TG production } (\mu\text{mol/min}) = \frac{\text{dose}}{\int_0^{\infty} SA(t) dt}$$

$$\text{VLDL-TG clearance (ml/min)} = \frac{\text{VLDL-TG production rate}}{C_{\text{VLDL-TG}}}$$

where *C*_{VLDL-TG} is the average VLDL-TG concentration during the basal period.

Residual VLDL-TG SA at *t* = 300 min (a measure of the injected labeled VLDL still not partitioned into oxidation or redeposition at the end of the basal period) was calculated as SA at *t* = 300 divided by the projected SA at *t* = 0 min (*A*₁ + *A*₂).

Adipose tissue VLDL-TG uptake. Fractional adipose tissue VLDL-TG uptake rate in upper and lower body subcutaneous fat was calculated using the regional (abdomen or thigh) adipose lipid SA (dpm/g dry wt) multiplied by the total amount of lipid (g) in that region divided by the injected dose. The VLDL-TG storage rates in specific regions were calculated as the fractional uptake times VLDL-TG production (μmol/min).

VLDL-TG oxidation. The ¹⁴CO₂ production rate from the oxidation of [1-¹⁴C]VLDL-TG (dpm/min) was corrected using the acetate recovery factor for resting conditions as reported by Sidossis et al.

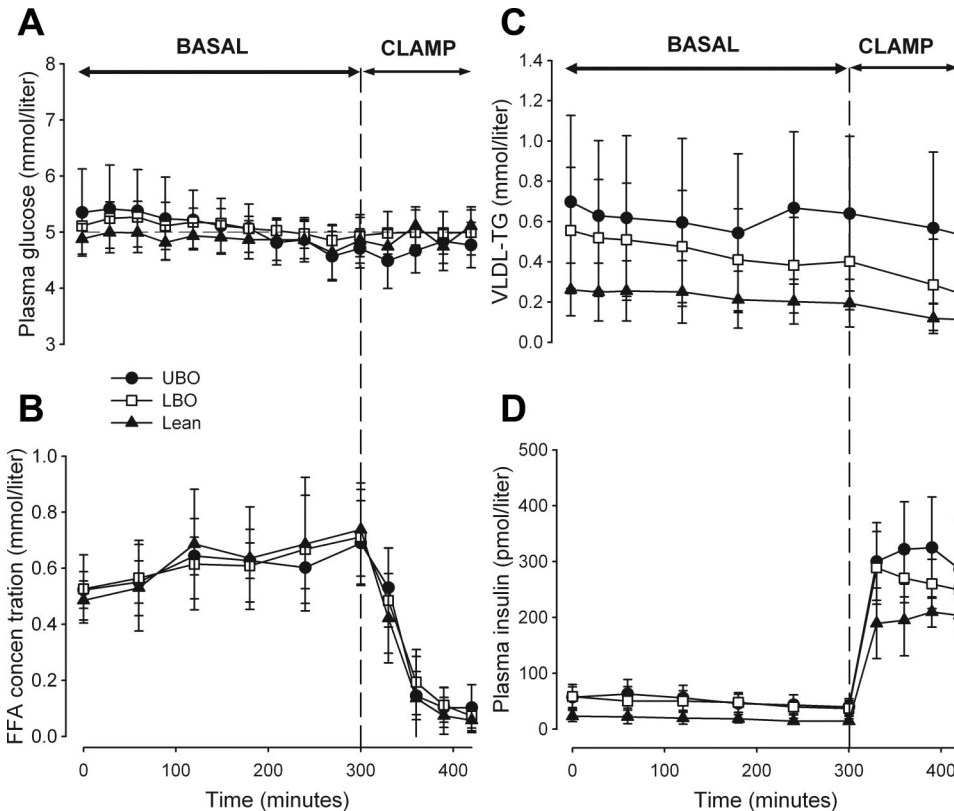


Fig. 1. Circulating plasma glucose (A), free fatty acid (FFA; B), VLDL-triglyceride (VLDL-TG; C), and insulin (D). ●, Upper body obese (UBO), *n* = 10 subjects; □, lower body obese (LBO), *n* = 10; ▲, lean, *n* = 10. Data are presented as means with SD.

(37) equal to 0.56. The total amount of [^{14}C]VLDL-TG oxidized (dpm) was calculated as the area under the curve ($\text{AUC}_{^{14}\text{C}_{\text{oxi}}}$) of dpm per minute vs. time, and the fractional VLDL-TG oxidation was calculated by dividing the corrected $\text{AUC}_{^{14}\text{C}_{\text{oxi}}}$ by the injected [^{14}C]VLDL-TG dose. VLDL-TG oxidation rate ($\mu\text{mol}/\text{min}$) was calculated as the fractional oxidation (%injected dose) times VLDL-TG production ($\mu\text{mol}/\text{min}$).

Statistics

Data were analyzed using SPSS for Windows version 14 (SPSS, UK, Chertsey, UK). Statistical significance was set at $P < 0.05$. All data are presented as means with SD as set out in guidelines for reporting statistics by the American Physiological Society (5). Comparisons of all three groups were made using ANOVA, and post hoc comparisons between subsets of groups were done by Tukey's honest significant difference test. All data sets were tested for a normal distribution according to the Kolmogorov-Smirnov test.

RESULTS

Subject Characteristics

Thirty volunteers (10 UBO, 10 LBO, and 10 lean women) completed the study. The two groups of obese women were well matched for age, BMI, lean body mass (LBM), fat mass, fat percent, circulating metabolites, and fasting insulin (Tables 1 and 2). The three groups did not differ on renal, hematologic, or liver indexes. By design, UBO subjects had greater WHR than both LBO and lean. Lean subjects differed from obese subjects on all anthropometric, biochemical, and hormonal parameters, except age, height, and FFA concentration.

Basal and Insulin-Stimulated Glucose and Lipid Metabolism

Basal glucose and lipid oxidation rates did not differ between groups (Table 2), whereas insulin-mediated glucose disposal was significantly reduced in both UBO and LBO women compared with lean women [GIR ($\text{mg}\cdot\text{kg}\cdot\text{LBM}^{-1}\cdot\text{min}^{-1}$): UBO: 3.9 (SD 1.6) vs. LBO: 4.5 (SD 2.6) vs. lean: 7.0 (SD 2.6), ANOVA, $P = 0.01$].

VLDL-TG Turnover

VLDL-TG concentrations, glucose, and FFAs remained constant throughout the basal period, enabling us to calculate VLDL-TG kinetics with precision (Fig. 1). As expected, hyperinsulinemia resulted in a near-complete suppression of cir-

culating FFAs, whereas VLDL-TG concentrations only decreased slightly in all groups.

As seen in Fig. 2A, VLDL-TG SA was well above the background level in all groups and significantly higher in lean subjects compared with both obese groups. The higher VLDL-TG SA in lean subjects was a consequence of comparable doses of tracer administered to subjects with varying VLDL-TG pool sizes. As depicted in Fig. 2B, the relative decay curves of the three groups were comparable. The decay fitted both a biexponential and a monoexponential decay, as shown in one representative subject (Fig. 2C). For all subjects, the biexponential function proved a better fit than a monoexponential decay. At $t = 300$ min, residual [^{14}C]VLDL-TG SA as percentage of activity at 0 min was as follows: UBO: 22% (SD 18) vs. LBO 15% (SD 11) vs. lean: 8% (SD 7), ANOVA, $P = 0.05$.

Figure 3, top, depicts VLDL-TG production rate, which was significantly greater in the UBO women compared with lean women. On the other hand, the VLDL-TG production rate in LBO women was not significantly different from UBO or lean women [VLDL-TG production ($\mu\text{mol}/\text{min}$): UBO: 64.8 (SD 40.0) vs. LBO: 42.5 (SD 25.6) vs. lean: 31.8 (SD 13.3), ANOVA, $P = 0.04$]. VLDL-TG clearance (ml/min) was comparable between groups.

VLDL-TG Adipose Tissue Uptake

Subcutaneous adipose tissue ^{14}C SA did not differ significantly between the three groups in the femoral or the abdominal region (data not shown). However, LBO women deposited a significantly larger amount of VLDL-TG in their lower-body subcutaneous fat depots than lean women [VLDL-TG redeposition in sc femoral adipose tissue ($\mu\text{mol}/\text{min}$): UBO: 5.1 (SD 3.1) vs. LBO: 5.8 (SD 4.3) vs. lean: 2.3 (SD 1.5), ANOVA, $P = 0.04$] (Fig. 4A). The uptake in subcutaneous femoral fat was not significantly different between LBO and UBO women or between UBO and lean women. Expressed in relative terms (%total VLDL-TG turnover), LBO women channeled a significantly greater proportion of VLDL-TG toward redeposition in subcutaneous femoral depots than both UBO and lean women (Fig. 4B). The relative subcutaneous femoral fat uptake was not significantly different between UBO and lean women. In the abdominal region, UBO women deposited significantly more

Table 1. Anthropometric indexes

	UBO	LBO	Lean	ANOVA (P value)
<i>n</i>	10	10	10	
Age, yr	42 (27–51)	38 (29–53)	39 (20–51)	0.57
Height, m	1.68 (0.06)	1.68 (0.03)	1.69 (0.04)	0.83
Weight, kg	91 (10)	86 (7)	66 (6)*†	<0.0001
BMI, kg/m^2	32.3 (2.3)	30.5 (1.8)	22.9 (1.4)*†	<0.0001
Waist-to-hip ratio	0.91 (0.05)	0.77 (0.04)*	0.78 (0.07)*	<0.01
Lean body mass, kg	51 (7)	47 (3)	42 (5)*	<0.0001
Fat mass, kg	36 (4)	36 (5)	19 (5)*†	<0.0001
Visceral fat mass, kg	4.8 (1.5)	4.0 (1.5)	2.3 (1.0)*†	<0.001
UB sc fat mass, kg	13.5 (2.6)	11.8 (2.4)	5.3 (1.9)*†	<0.0001
LB sc fat mass, kg	12.9 (1.6)	14.9 (1.9)*	8.6 (2.1)*†	<0.0001
Fat, %	41 (3)	42 (2)	30 (6)*†	<0.0001

All data are presented as means with SD or mean (range); *n*, no. of subjects. UBO, upper body obese; LBO, lower body obese; BMI, body mass index; UB, upper body; LB, lower body; sc, subcutaneous. Symbols in the table refer to post hoc comparisons between groups (Tukey's test): $P < 0.05$ vs. UBO (*) and vs. LBO (†).

Table 2. Circulating metabolites, hormones, and metabolic parameters

	UBO	LBO	Lean	ANOVA (P Value)
<i>n</i>	10	10	10	
Insulin, pM	60 (16)	58 (22)	23 (10)*†	<0.001
β-Glucose, mM	5.4 (0.7)	5.1 (0.3)	4.9 (0.3)	0.05
HbA _{1c} , %	5.5 (0.3)	5.5 (0.3)	5.4 (0.2)	0.72
FFA, mM	0.55 (0.11)	0.52 (0.07)	0.48 (0.07)	0.24
TG, mM	1.06 (0.50)	0.99 (0.35)	0.65 (0.28)	0.06
VLDL-TG, mM	0.63 (0.39)	0.46 (0.27)	0.23 (0.13)*	0.01
Total cholesterol, mM	4.5 (0.6)	4.9 (0.9)	5.1 (0.9)	0.23
HDL cholesterol, mM	1.3 (0.3)	1.9 (0.6)*	2.0 (0.4)*	0.01
LDL cholesterol, mM	2.3 (0.7)	2.5 (1.0)	2.7 (0.8)	0.53
Glucose oxidation, mg·kg ⁻¹ ·min ⁻¹	1.05 (0.17)	1.09 (0.38)	1.36 (0.63)	0.28
Lipid oxidation, mg·kg ⁻¹ ·min ⁻¹	0.71 (0.18)	0.65 (0.11)	0.65 (0.23)	0.74

All data are presented as means with SD or mean (range); *n*, no. of subjects. FFA, free fatty acid; TG, triglyceride; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Symbols refer to post hoc comparisons between groups (Tukey's test): *P* < 0.05 vs. UBO (*) and vs. LBO (†).

VLDL-TG than lean women expressed in absolute terms (μmol/min), whereas the absolute uptake was not significantly different between LBO and UBO women or between LBO and lean women [VLDL-TG redeposition in abdominal adipose tissue (μmol/min): UBO: 5.0 (SD 2.9) vs. LBO: 4.0 (SD 3.2) vs. lean: 1.3 (SD 1.0), ANOVA, *P* = 0.01] (Fig. 4C). In relation to VLDL turnover (%), however, both UBO and LBO women channeled a significantly greater proportion of VLDL-TG toward deposition in abdominal subcutaneous fat compared with lean women (Fig. 4D). The relative VLDL-TG uptake in subcutaneous abdominal fat was not significantly different between UBO and LBO women.

VLDL-TG Oxidation

Basal VLDL- and TG-derived fatty acid oxidation rates in the three groups are depicted in Fig. 4, E and F. The proportion of total VLDL-TG turnover that was oxidized was comparable in each of the three groups. As a result, the absolute oxidation rate of the UBO women was somewhat greater compared with LBO and lean women, however, not significantly so [VLDL-TG oxidation rate (μmol/min): UBO: 38.3 (SD 26.5) vs. LBO: 23.5 (SD 13.5) vs. lean: 21.1 (SD 9.7), ANOVA, *P* = 0.09] (Fig. 4E). VLDL-TG constituted a significant source of energy, as evidenced by oxidation rates [VLDL-TG oxidation rate

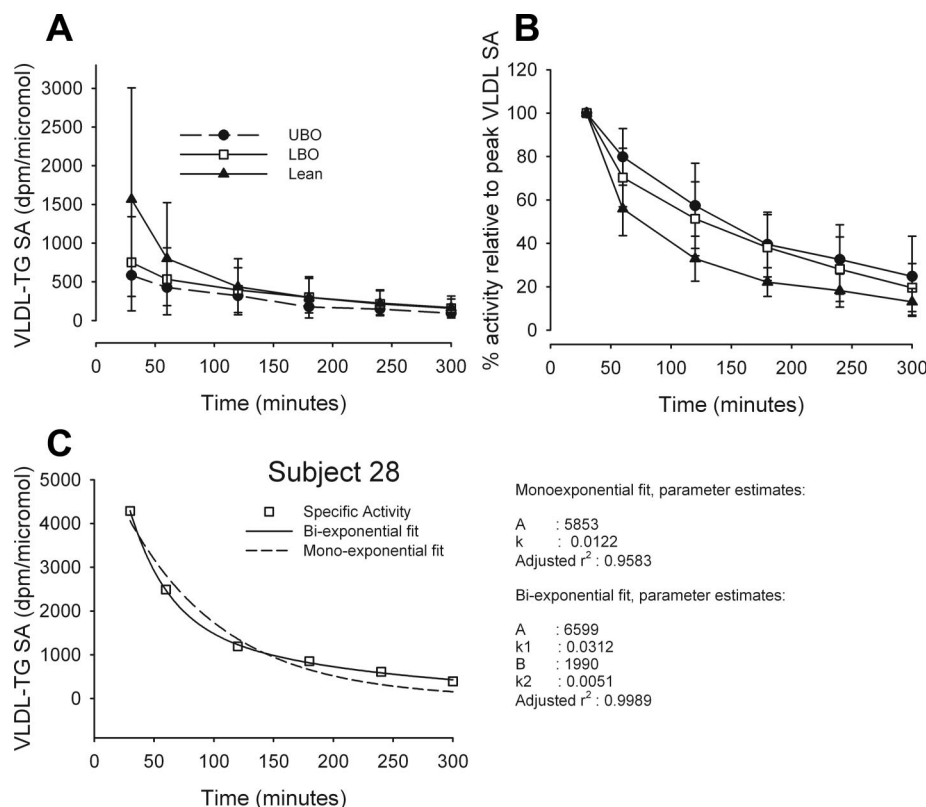


Fig. 2. A: very-low-density lipoprotein (VLDL)-TG specific activity (SA) expressed in absolute terms [disintegrations·min⁻¹ (dpm)·μmol VLDL-TG⁻¹]. As seen, SA was markedly higher in lean subjects than in LBO or UBO because of their smaller VLDL-TG pool. B: VLDL-TG SA expressed in relative terms (%peak activity). In all subjects, peak activity occurred at the earliest point measured and decreased in a biexponential manner to levels ~20% after 5 h. C: comparison of a monoexponential and biexponential fit to the VLDL-TG SA vs. time curve in a representative subject (LBO). For the entire study population, the biexponential fit provided significantly better *r*² than the monoexponential fit [mono: 0.91 (SD 0.10) vs. bi: 0.94 (SD 0.11), *P* = 0.04]. Data are presented as means with SD.

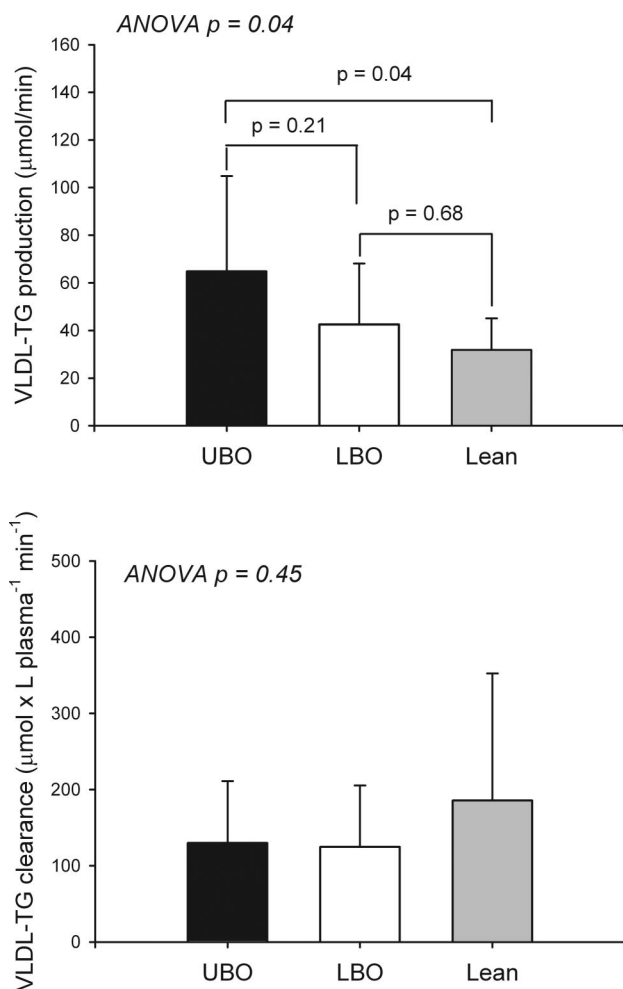


Fig. 3. *Top*: obesity phenotype had a significant impact on VLDL-TG production, with UBO women producing more than lean women. Post hoc comparisons between groups were assessed by Tukey's multiple-comparison test. *Bottom*: no difference in VLDL-TG clearance was observed between groups although a tendency toward a greater clearance was observed in the lean group when compared with both obesity phenotypes. Data are presented as means with SD.

(kcal/min): UBO: 0.30 (SD 0.21) vs. LBO: 0.18 (SD 0.11) vs. lean: 0.17 (SD 0.08), ANOVA, $P = 0.09$]. Because a proportion of VLDL- and TG-derived fatty acids are oxidized in skeletal muscle, we also compared the three groups after normalizing for LBM. However, this only reduced the difference between groups.

DISCUSSION

Variations in the metabolic fate of VLDL-TG may have impacts on a number of pathways, but, until recently, it has not been possible to study the disposition of the fatty acids in VLDL-TG. In this study, we report that, although we found hepatic VLDL-TG output to be approximately two times as great in UBO women compared with lean women, only a slightly larger amount was found to be channeled toward redeposition in subcutaneous fat. In addition, LBO women were able to direct a greater proportion of VLDL-TG turnover toward deposition in both UBSC and femoral fat, whereas, in UBO women, this proportion was greater only in UBSC fat.

Furthermore, the oxidation rate of VLDL- and TG-derived fatty acids accounted for the major part of VLDL-TG removal but was not significantly different between obesity phenotypes. To our knowledge, this is the first report of how VLDL-TGs are trafficked toward oxidation and storage in subcutaneous fat in humans.

How body composition impacts on VLDL-TG kinetics has been the focus of some previous studies. Mittendorfer et al. (24, 26) reported increased levels of circulating VLDL-TG but similar VLDL-TG production rates in women with abdominal obesity compared with lean subjects. Earlier, Reaven and Bernstein (32) also reported comparable VLDL-TG production rates in obese and lean women despite elevated levels of circulating TGs. By contrast, we report both greater VLDL-TG production rates and VLDL-TG concentrations in UBO women than in lean subjects. There are a number of reasons supporting the notion that the location of fat depots may affect hepatic secretion of VLDL-TG. First, FFAs from visceral fat lipolysis drains in the portal vein, and excess FFAs released from large fat depots may therefore serve as ample substrate for reesterification in hepatocytes. Whereas some of the newly synthesized TGs are stored in the hepatocytes causing hepatic steatosis (7), approximately one-fifth of the FFAs entering the liver are secreted as VLDL-TG (12). Second, preferential weight loss from abdominal depots results in decreased VLDL-TG production of which a large proportion has been demonstrated to originate from viscera-derived fatty acids (25). Third, there is evidence to suggest that hepatic insulin resistance may arise as a consequence of elevated FFAs (2). Because insulin suppresses VLDL-TG production (16, 20), ablation of this inhibitory effect may result in increased VLDL particle assembly and subsequent VLDL-TG secretion. Fourth, it has been demonstrated recently using the nuclear magnetic resonance technique that viscera-obese individuals secrete more and larger VLDL particles than individuals with preferential subcutaneous fat accumulation (34). Because large VLDL particles contain more TG than smaller particles, we find our observation of greater VLDL-TG secretion in the UBO women in line with these new data.

After estimating basic VLDL-TG kinetic parameters, we assessed VLDL-TG trafficking toward redeposition in adipose tissue using fat biopsies. At the endothelial surface, FFAs are released from VLDL-TG by LPL activity and subsequently channeled into either skeletal muscle or redeposited in adipose tissue. The adipose tissue-to-skeletal muscle ratio is higher in obese than in lean subjects, and, assuming that VLDLs flow freely in all capillary beds, the fraction of an injected VLDL-bound tracer that ultimately ends up in adipose tissue is therefore likely to be greater in obese women than in lean subjects. This was also the case in the present study in which both LBO and UBO women redeposited a rather small but significantly larger amount of VLDL- and TG-derived fatty acids in abdominal subcutaneous tissue than lean women. In the femoral subcutaneous tissue, LBO women deposited more than lean women, probably as a consequence of their larger storage capacity in the region. On the whole, both groups of obese women channeled a larger fraction of the injected dose toward redeposition in subcutaneous adipose tissue than did lean women. However, the absolute amount of VLDL-TG partitioned into redeposition in our obese subjects only constituted ~ 10 $\mu\text{mol}/\text{min}$, corresponding to $\sim 16\%$ of total

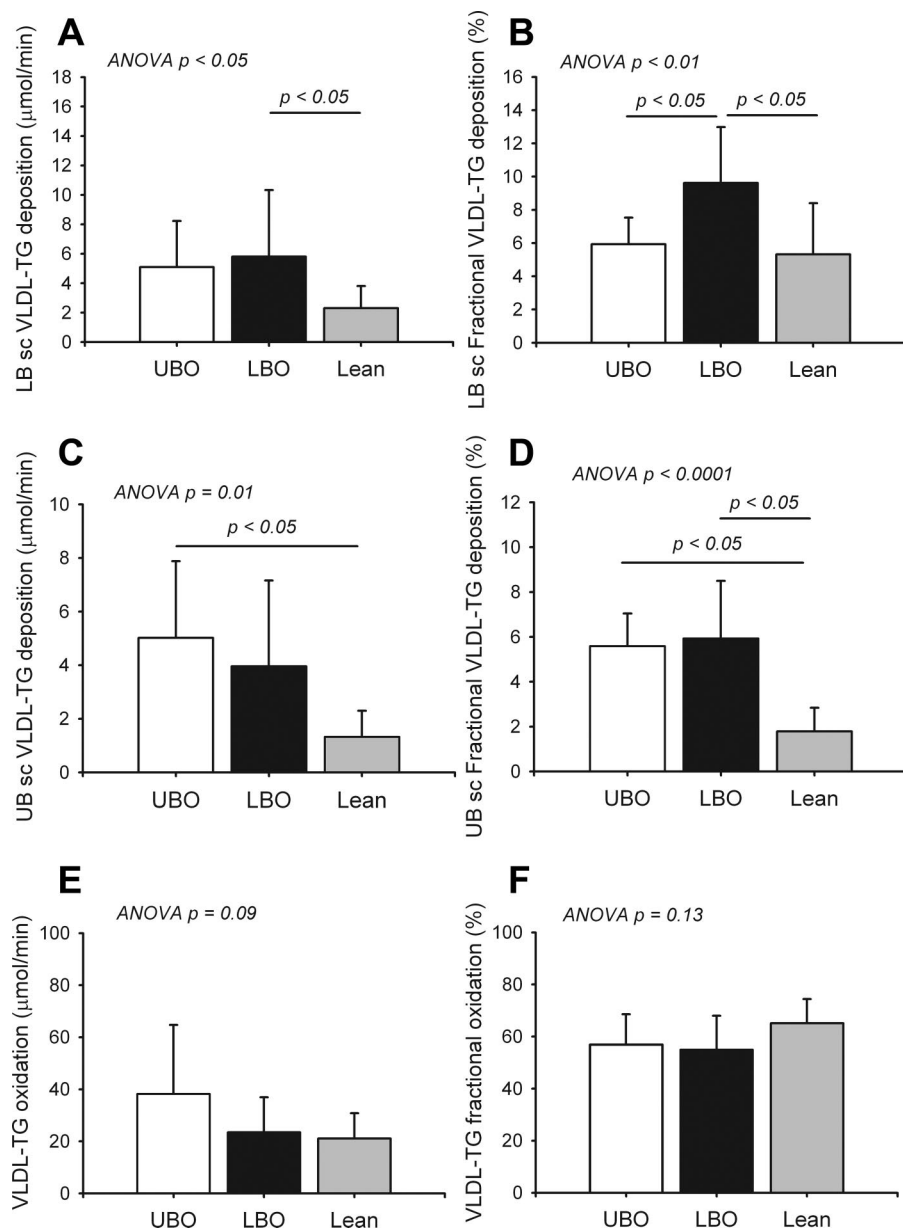


Fig. 4. Fate of [¹⁴C]VLDL-TG. A, C, and E: VLDL-TG channeling (μmol/min) into either oxidation (A), redeposition in abdominal adipose tissue (C), or femoral adipose tissue (E). B, D, and F: fractional VLDL-TG oxidation (%) and redeposition; n = 30. Data are presented as means with SD. Post hoc comparisons between groups were assessed by Tukey's multiple-comparison test.

VLDL-TG production. Our data thus indicate that hepatic overproduction of VLDL-TG cannot be readily absorbed into adipose depots.

Finally, we investigated whether differences in oxidation of VLDL-derived fatty acids exist between women of different obesity phenotypes. To our knowledge, this question has not previously been addressed because of considerable methodological obstacles. We are only aware of few studies in which it has been attempted to directly measure VLDL-derived fatty acid oxidation [the elegant studies by Sidossis et al. (39) using in vivo labeling of the VLDL-TG moiety by [¹³C]glycerol followed by plasmapheresis]; however, subjects enrolled in that series of studies were not comparable with our study population. In the present study, VLDL-TG oxidation accounted for ~20% of our subjects' daily energy requirements, suggesting that circulating triglycerides constitute an important source of lipid fuels during short-term fasting. However, no

differences in fractional or absolute VLDL-TG oxidation between lean and obese women were observed, although UBO tended to oxidize more VLDL-TG (in μmol/min) than both LBO and lean subjects. Because both lipid availability and the oxidative capacity, in the form of LBM, were greater in UBO women than in lean or LBO women, absolute VLDL-TG oxidation rates could be anticipated to be greater in UBO women than in lean women. That no such difference could be found may suggest that UBO women are not as metabolically flexible as lean or LBO women. This notion is further corroborated by our UBO subjects' relative insulin resistance compared with the other two groups.

Given the unsuitability of previous VLDL-TG kinetic studies to trace the final fate of VLDL-derived fatty acids, we chose to use a recently validated [1-¹⁴C]triolein VLDL-TG tracer (10) that has been demonstrated to offer reliable estimates of tracer incorporation in adipocytes (by fat biopsies) or oxidation

(by $^{14}\text{CO}_2$ production). This approach allowed us to directly measure the partitioning of VLDL-associated fatty acids (in the form of [^{14}C]oleate) in either oxidative or storage pathways. Our choice of the [^{14}C]triolein VLDL tracer also allowed us to calculate VLDL-TG production rates without estimating the total VLDL-TG pool. Traditionally, VLDL-TG has been thought to be confined solely to the blood stream, and the total VLDL-TG pool has therefore been estimated to constitute plasma volume multiplied by VLDL-TG concentration. Total VLDL-TG production rates have then been calculated as the fractional catabolic rate (FCR) multiplied by the VLDL-TG pool, where the FCR has been calculated in a number of ways using either multicompartmental modeling (1, 42) or bolus injection of a precursor and estimation of the subsequent labeled VLDL-TG decay slope (3, 18). However, this approach has consistently yielded lower estimates of total VLDL-TG production than more direct methods like, e.g., splanchnic balances (12) or the primed-constant infusion technique with in vivo labeled VLDL-TG (38), both methods that are independent of VLDL-TG pool estimates. Taken together, this suggests either that calculated FCRs have been too low (which may very well be the case when injection of a precursor has been the tracer of choice) or that the total VLDL-TG pool in which the tracer dissolves has been underestimated. On average, we found VLDL-TG apparent volume of distribution to be approximately twofold higher than calculated solely from plasma volume (data not shown). That the VLDL-TG apparent volume of distribution has been underestimated in the past is supported by the present study, since a biexponential decay curve invariably fit our experimental data better than a mono-exponential fit. Thus, judging from our decay curves, a second VLDL-TG pool that is not directly accessible appears to exist. Whether this second pool represents endothelial receptor-bound VLDL, extravasated VLDL particles, or an intrahepatic pool cannot be determined by these experiments. However, our estimates of VLDL-TG production in lean subjects were comparable with those obtained by the primed-constant infusion technique (27, 39) or splanchnic balances (12), but three- to fivefold higher than those obtained using techniques relying on an estimate of the VLDL-TG pool (3, 11, 24, 28). That VLDL-TG pool size impacts profoundly on calculated VLDL-TG production rates has previously been reviewed extensively by Magkos and Sidossis (22).

An important limitation to the present study must be acknowledged. To study whether fat mass per se or fat location determines VLDL-TG production and secretion, only women were considered for inclusion in the study. This approach was chosen since both LBO and viscerally obese women are frequent in the Danish population. By contrast, obese men are almost always viscerally obese, and recruiting a male population spanning the same range of body composition would therefore have been difficult. When interpreting the results of our data, one must therefore take into consideration that sex hormones could also influence VLDL-TG turnover.

In summary, the hypertriglyceridemia of UBO women is caused by hepatic VLDL-TG overproduction without concomitant increased removal via oxidation and adipose tissue redeposition. In addition, evaluation of our labeled VLDL-TG decay curves leads us to propose that a second VLDL-TG compartment exists. Last, our data suggest that circulating triglycerides constitute a significant energy source in the post-

absorptive state. To our knowledge, this is the first comprehensive overview of VLDL-TG metabolism, including removal mechanisms via either redeposition or oxidation.

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