

Energy expenditure, insulin, and VLDL-triglyceride production in humans

Lars C. Gormsen,^{*} Michael D. Jensen,[†] Ole Schmitz,^{*,§} Niels Møller,^{*} Jens S. Christiansen,^{*} and Søren Nielsen^{1,*}

Medical Department M (Endocrinology and Diabetes),^{*} Aarhus University Hospital, DK-8000 Aarhus, Denmark; Endocrine Research Unit,[†] Mayo Clinic, Rochester, MN 55905; and Department of Clinical Pharmacology,[§] University of Aarhus, DK-8000 Aarhus, Denmark

Abstract Hypertriglyceridemia is considered a cardiovascular risk factor in diabetic and nondiabetic subjects. In this study, we aimed to determine potential regulators of very low density lipoprotein-triglyceride (TG) production. VLDL-TG kinetics were measured in 13 men and 12 women {body mass index [mean (range)]: 24.8 (20.2–35.6) kg/m²}. VLDL-TG production was assessed from the plasma decay of a bolus injection of ex vivo labeled VLDL particles ([1-¹⁴C]triolein-VLDL-TG). Similar VLDL-TG production (μmol/min) was found in men and women. VLDL-TG production was not significantly correlated with palmitate flux ([9,10-³H]palmitate) ($r = 0.09$, $P = 0.67$) or palmitate concentration ($r = -0.29$, $P = 0.2$) but was correlated significantly with fasting insulin concentration ($r = 0.46$, $P < 0.05$) and resting energy expenditure (REE) ($r = 0.45$, $P < 0.05$). The latter correlation improved when adjusted for sex. The best multivariate model with VLDL-TG production as the dependent variable and REE, body composition, hormones, and substrate levels as independent variables included fasting insulin ($P = 0.02$) and REE ($P = 0.02$) ($r^2 = 0.32$, $P < 0.001$). We conclude that VLDL kinetics are similar in men and women and that REE and plasma insulin are significant independent predictors of VLDL-TG production. FFA availability and body fat distribution are unrelated to VLDL production. We suggest that REE plays a greater role in VLDL-TG production than previously anticipated. REE and insulin should be taken into account when VLDL-TG production comparisons between groups are made.—Gormsen, L. C., M. D. Jensen, O. Schmitz, N. Møller, J. S. Christiansen, and S. Nielsen. **Energy expenditure, insulin, and VLDL-triglyceride production in humans.** *J. Lipid Res.* 2006. 47: 2325–2332.

Supplementary key words very low density lipoprotein • lipoproteins • free fatty acids

An increased concentration of plasma triglyceride (TG) is recognized as an independent cardiovascular risk factor (1) and is a frequent finding in insulin-resistant conditions such as obesity and type 2 diabetes. Although plasma TG

concentration is a well-described parameter in health and disease, our knowledge about plasma TG kinetics and factors affecting TG metabolism is still limited. This is probably attributable to the lack of easily available, robust methods to determine TG production, as indicated by the use of a wide variety of approaches (2).

In the postabsorptive state, circulating TGs are found predominantly in VLDL particles. The absolute and relative turnover of this TG pool determines the plasma TG concentrations, reflecting the balance between the hepatic secretion and peripheral clearance of VLDL-TG. The latter event is largely determined by endothelial lipoprotein lipase activity and receptor-mediated VLDL uptake. Among factors proposed to regulate VLDL-TG metabolism is FFA availability (3, 4), which has long been considered a key regulator of VLDL production. However, fasting FFA availability is in turn influenced by other factors, such as resting energy expenditure (REE) (5), body composition (5–7), catecholamine availability (5, 8–11), and sex (5). Fasting plasma insulin has also been reported to have an effect on VLDL metabolism (9), and it has been demonstrated that hyperinsulinemia caused by massive carbohydrate ingestion is associated with increased VLDL-TG production independent of the concomitant increase in FFA levels (8). On the other hand, an acute exposure to high levels of insulin decreases VLDL-TG output by the liver (10).

Recently, we reported significantly greater FFA (palmitate) turnover in women compared with men and a strong relationship between FFA turnover and REE (5). The greater FFA turnover in women was noted not only at comparable levels of REE and relative oxidation rates of glucose and lipids but also at similar plasma FFA and TG concentrations. These findings can only be explained by an increased nonoxidative clearance of FFAs in women compared with men. One nonoxidative mechanism by which FFAs can be readily cleared from the circulation is by the hepatic production and subsequent release of

Manuscript received 19 April 2006 and in revised form 13 July 2006.

Published, *JLR Papers in Press*, July 18, 2006.
DOI 10.1194/jlr.M600175-JLR200

¹To whom correspondence should be addressed.
e-mail: nielsen.soren@dadlnet.dk

Copyright © 2006 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at <http://www.jlr.org>

VLDL-TG particles into the circulation destined for adipose tissue storage. Whether women channel more FFAs toward VLDL-TG than men and whether a relationship with REE also exists for VLDL-TG is unknown. This study was designed to further investigate differences in VLDL turnover in men and women. We specifically wanted to establish whether a relationship exists between REE and VLDL-TG production and, if so, to what extent this relationship was modified by sex, FFA availability, body composition, and hormone levels. If a significant relationship between VLDL-TG production and REE can be demonstrated, perhaps independent of FFA availability, it would change our present perception of VLDL-TG from serving merely as a passive transporter of TG for adipose tissue storage toward an additional direct role of VLDL particles in substrate delivery to energy-consuming tissues. We used a recently validated method involving *ex vivo* labeling of native VLDL-TG with subsequent calculation of VLDL-TG production based on the monoexponential decay of VLDL-TG specific activity (SA) (12).

RESEARCH DESIGN AND METHODS

Subjects

Twenty-six healthy subjects (13 women and 13 men) were included in the study. All participants were nonsmokers, not taking any medication, and had been weight-stable for the previous 3 months. All were normotriglyceridemic and had normal blood pressure, normal hematological indices, and normal liver and renal function. All women were premenopausal. Subjects were recruited such that a wide range of body fat distribution was included among both sexes. Informed consent was obtained from all participants, and the Ethics Committee gave its approval. One woman had to be excluded during the study because of technical difficulties.

Body composition

Dual-energy X-ray absorptiometry (QDR-2000; Hologic, Inc., Waltham, MA) and computed tomography of the L₂₋₃ interspace (13) was performed in the days preceding the examination to determine the body composition [total fat mass (FM) and fat-free mass (FFM)] of the participants.

Indirect calorimetry

REE and substrate oxidation rates (14) were measured by indirect calorimetry (Deltatrac monitor; Datex Instrumentarium, Helsinki, Finland). The initial 5 min was used for acclimatization to the hood, and REE was then measured from 65 to 90 min.

Palmitate turnover

Systemic palmitate flux was measured at 30–60 min using the isotope dilution technique with a constant infusion of [9,10-³H]palmitate (0.3 μCi/min) (Lægemedelstyrelsen, Copenhagen, Denmark) (15) from 0 to 60 min. Blood samples for the measurement of palmitate concentration and SA were drawn at baseline and at 10 min intervals over the last 30 min of the infusion. The steady-state SA was verified (30, 40, 50, and 60 min) for each individual.

Plasma palmitate concentration and SA were determined by HPLC using [²H₃₁]palmitate as the internal standard. Systemic

palmitate flux (μmol/min) was calculated using the [9,10-³H]palmitate infusion rate (dpm/min) divided by the steady-state palmitate SA (dpm/μmol).

VLDL-TG concentration and VLDL-TG SA

VLDL was isolated from plasma by ultracentrifugation. Approximately 3 ml of each plasma sample was transferred into Optiseal tubes (Beckman Instruments, Inc., Palo Alto, CA), covered with a saline solution (*d* = 1.006 g/ml), and centrifuged (50.3 rotor; Beckman Instruments) for 18 h at 40,000 *g* and 10°C. The top layer, containing VLDL, was aspirated, and the exact volume was recorded. A small proportion was analyzed for TG content, and the plasma concentration of VLDL-TG was calculated. This procedure results in VLDL-TG concentrations that are highly correlated with total plasma TG concentration (*r* = 0.86, *P* < 0.000001). However, because the aspiration of VLDL particles is not complete, VLDL-TG concentrations are slightly underestimated. The remaining VLDL-TG was transferred to a scintillation glass vial, 10 ml of scintillation liquid was added (Optiphase HiSafe 2; Wallac), and the sample was measured for ¹⁴C activity using dual-channel counting.

VLDL tracer preparation

One week before the examination, a 40 ml blood sample was obtained aseptically from each participant. The VLDL fraction was separated from plasma as described above and transferred to a sterile glass test tube containing 20 μCi of [1-¹⁴C]triolein dissolved in 200 μl of ethanol. The solution was then gently sonicated in a water bath at 37°C for 30 min and subsequently filtered through a sterile 20 μm filter (Filtropur). A 300 μl sample of the solution was tested for bacterial growth to rule out contamination during the process.

To ensure that [1-¹⁴C]triolein was incorporated into the lipoprotein particles, each sample was assayed by size-exclusion HPLC as described previously (12). An HPLC radiochromatogram demonstrated that the radioactivity coeluted with VLDL-sized particles, indicating successful incorporation of radioactive tracer into the VLDL lipoproteins.

Calculations

Because VLDL particles are essentially confined to plasma, the VLDL-TG pool can be viewed as a single compartment system. Therefore, the decay of SA in radiolabeled VLDL-TG after a bolus injection is monoexponential and can be described using the following general equation:

$$SA_t = SA_{peak} \times e^{-FCR \times t}$$

where SA_t = SA of VLDL-TG (dpm/μmol) at time *t*, SA_{peak} = SA of VLDL-TG at the peak of enrichment, FCR = fractional catabolic rate (pools/min), and *t* = time (min).

The FCR of the VLDL-TG pool is easily calculated from the slope of the log SA (dpm/μmol VLDL-TG) versus time curve as described previously (16):

$$FCR = - \left(\frac{\ln SA_t - \ln SA_{peak}}{t} \right)$$

Because the VLDL tracer could potentially be “contaminated” by small amounts of free [1-¹⁴C]triolein, the slope was calculated from 120 min onward. This time point was chosen because control experiments performed in our laboratory have shown that any free [1-¹⁴C]triolein injected at time 0 has been cleared from the circulation and contributes in a nominal way to the VLDL SA decay curve after 2 h (12).

VLDL-TG kinetics were computed as follows:

$$\text{VLDL-TG production rate } (\mu\text{mol}/\text{min}) = \text{FCR}/60 \times C_{\text{VLDL-TG}} \times \text{PV}$$

$$\text{VLDL-TG secretion rate } (\mu\text{mol}/\text{L plasma}/\text{min}) = \text{FCR}/60 \times C_{\text{VLDL-TG}}$$

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

where $C_{\text{VLDL-TG}}$ = average VLDL-TG concentration from time 0 to 300 min and PV = plasma volume. Plasma volume was calculated based on each participant's FFM (17).

$$\text{PV} = 0.055 \times \text{FFM}$$

Assays

Serum growth hormone was analyzed with a double monoclonal immunofluorometric assay (Delfia; Wallac Oy, Turku, Finland). Serum insulin was measured with an immunoassay (DAKO). Serum FFA was determined by a colorimetric method using a commercial kit (Wako Pure Chemical Industries, Ltd., Neuss, Germany). Epinephrine and norepinephrine were measured by HPLC. Plasma TG concentration was analyzed using a COBAS Fara II.

Sample collection

Blood samples were collected in chilled tubes and placed in an ice bath, and plasma was then separated as quickly as possible by centrifugation (3,600 rpm at 4°C for 10 min). Aliquots of plasma (3 ml) were refrigerated at 4°C for isolation of VLDL upon completion of the examination. The remaining samples were stored at -20°C for later analysis.

Experimental protocol

One week before the start of the study, the participants were instructed by a clinical dietician to consume a weight-maintaining diet and to refrain from physical exercise for 3 days before examination. A blood sample was drawn to ensure that the participants had normal values for hematological, liver, and renal indices. A second blood sample was drawn to isolate and label VLDL. All female participants had a negative pregnancy test.

On the evening preceding the study day, subjects were admitted to the research laboratory at 11:00 PM and fasted overnight. Before the examination, they were allowed to use the toilet and were then allowed to either sit or lie in bed wearing light hospital clothing in a room with ambient temperature of 22–24°C. They remained in bed throughout the study. Two intravenous catheters (Venflon; Viggo AB, Helsingborg, Sweden) were inserted, one in an antecubital vein of the left arm and the other in a dorsal hand vein on the right. The hand was then placed in a heated box at 65°C, allowing for arterialized blood samples to be drawn. At 8:00 AM (time 0), after drawing baseline blood samples (insulin, TG, catecholamines, growth hormone), ^{14}C -labeled VLDL was administered as a bolus injection over 10 min. At the same time, a constant infusion of [^3H]palmitate (0.3 $\mu\text{Ci}/\text{min}$) was initiated and maintained for 1 h. Blood samples were drawn at 0, 30, 40, 50, and 60 min and analyzed for palmitate concentration and SA, insulin, growth hormone, and catecholamines. At 0, 30, 60, 120, 180, 240, and 300 min, blood samples were drawn and analyzed for VLDL-TG SA and total TG concentration. Indirect calorimetry was performed between 30

and 60 min. After completion of the study, intravenous catheters were removed and the participants were dismissed.

Statistics

All data are expressed as means \pm SEM unless stated otherwise. Variables that were not normally distributed were log-transformed before statistical processing. Between-group differences were analyzed using Student's *t*-test or the Mann-Whitney two-samples test. Correlations were evaluated by Pearson's *r*. Two-way ANOVA was performed to test the effect of sex and time on VLDL-TG concentration during the investigation. Univariate correlation analysis was used to identify variables that potentially affected VLDL-TG production (see Table 2 below). Accordingly, variables displaying the strongest univariate correlation ($P < 0.25$) with VLDL-TG production (REE and insulin level) were selected for consideration in a multivariate linear regression analysis to assess significant determinants of VLDL-TG production. In addition to REE and insulin, the following candidate independent variables were also considered: palmitate flux, FM, visceral fat area, and sex. Palmitate concentration was also entered instead of palmitate flux in the analysis. Using the approach of stepwise variable selection (stepping up), we accepted variables with a significance level of 0.1 or less into the final model. Because of the well-known colinearity between REE and FFM and because FFM is used in the equation to calculate VLDL production, models in which only REE and FFM were included were analyzed first. In both non-stepped and stepped multiple linear regression analyses, REE consistently emerged as a significant independent predictor of VLDL-TG production, whereas FFM was consistently eliminated. The latter was also true when FFM was forced into the model before REE. Thus, REE turned out to be a stronger determinant of VLDL-TG production despite the fact that FFM is used in the calculation of VLDL-TG production. Therefore, FFM was not included in the final models. To ensure that FM, palmitate flux, and palmitate concentration were not prematurely excluded from consideration, they were forced separately into nonstepwise models that also included the variables found to be significant in the stepwise model. Differences were considered significant at $P < 0.05$.

RESULTS

Clinical characteristics, body composition, and hormone concentrations

The mean and range of body mass index values of our volunteers are given in **Table 1**. The expected differences in FFM, FM, and percentage body fat between men and women were present. There were no statistically significant differences in intra-abdominal fat volumes between men and women, but women had significantly greater abdominal subcutaneous fat volume. REE was significantly greater in men than in women; however, the respiratory exchange ratio was not different, indicating similar relative fuel oxidation rates in men and women.

Hormones

Plasma norepinephrine, human growth hormone, and insulin concentrations measured at time 0 were comparable in men and women, whereas plasma epinephrine concentrations were significantly greater in women than in men (Table 1). Because epinephrine is a known stimulator

TABLE 1. Subject characteristics

Characteristic	Men (n = 13)	Women (n = 12)	P
Age (years)	25.9 ± 1.0	24.7 ± 1.6	NS
Body mass index (kg/m ²)	23.8 (21.1–29.8)	23.8 (20.2–35.6)	NS
Plasma volume (liters)	3.55 ± 0.99	2.42 ± 0.80	<0.0001
Fat-free mass (kg)	64.5 ± 1.8	44.1 ± 1.5	<0.0001
Total fat mass (kg)	12.3 (5.1–20.4)	17.9 (12.1–49.1)	0.01
Body fat (%)	15.2 ± 1.6	31.4 ± 2.8	<0.0001
Visceral fat area (cm ²)	93 ± 14	75 ± 17	NS
Subcutaneous fat area (cm ²)	65 (31–139)	101 (61–395)	<0.05
REE (kcal/24 h)	1836 ± 37	1438 ± 48	<0.0001
Respiratory exchange ratio	0.85 ± 0.01	0.87 ± 0.01	NS
Growth hormone (ng/ml)	0.93 (0.04–15.93)	0.83 (0.01–7.81)	NS
Insulin (pmol/l)	24 (13–48)	26 (14–87)	NS
Epinephrine (pg/ml)	31.5 (25–67)	65 (25–222)	<0.05
Norepinephrine (pg/ml)	128 (110–283)	172 (119–339)	NS

REE, resting energy expenditure. Data are means ± SEM or median and (range).

of hormone-sensitive lipase, the principal enzyme responsible for adipocyte FFA release, correlations between epinephrine and FFA concentration (palmitate), FFA turnover (palmitate flux), and VLDL-TG production were analyzed subsequently. However, no significant correlations between epinephrine concentration and palmitate concentration ($r = -0.09$, $P = 0.64$), palmitate flux ($r = 0.08$, $P = 0.70$), or VLDL-TG production ($r = 0.01$, $P = 0.96$) were observed; therefore, epinephrine was not considered for inclusion in the final multivariate regression model.

VLDL-TG kinetics

Plasma total TG concentration and VLDL-TG concentration remained constant throughout the study (Fig. 1, bottom panel), with no sign of a sex or time effect. The rate of decline in VLDL-SA is shown in Fig. 1, top panel. VLDL-SA was significantly higher in women compared with men but declined similarly in both sexes. Thus, no significant difference was observed for VLDL-TG FCR, VLDL-TG production, or VLDL-TG clearance (Table 2).

Because the initial multiple regression analysis (see below) revealed independent effects of REE and plasma insulin, these factors were plotted against VLDL-TG production. The top panel of Fig. 2 depicts the relationship between VLDL-TG production and REE. For the entire group, there was a significant positive correlation between VLDL-TG production and REE ($r = 0.45$, $P = 0.02$). Adjusting for sex, however, displayed two parallel regression slopes steeper than the combined slope (men, $r = 0.57$, $P = 0.043$; women, $r = 0.58$, $P = 0.048$) with significantly different y intercepts ($P < 0.01$). The relationship between VLDL-TG production and fasting plasma insulin was also significant. To better appreciate the apparent sex effect shown in the upper panel plot (VLDL-TG production vs. REE), VLDL-TG production was plotted against the residuals derived from the linear regression of VLDL-TG production on REE (Fig. 2, bottom panel). It appears that the effect of insulin is independent of a sex effect. One woman diverged markedly from the remaining subjects (upper left corner of the plot). However, exclusion of this subject could not be justified.

Table 3 shows the univariate correlations between VLDL-TG production and the candidate independent predictors. Based on a cutoff value of $P < 0.25$, we identified the determinants most likely to have a major impact on VLDL-TG production to include them in a multivariate regression analysis. Because sex changed the intercept value between REE and VLDL-TG markedly (Fig. 2), it was also included in the final model. Tables 4 and 5

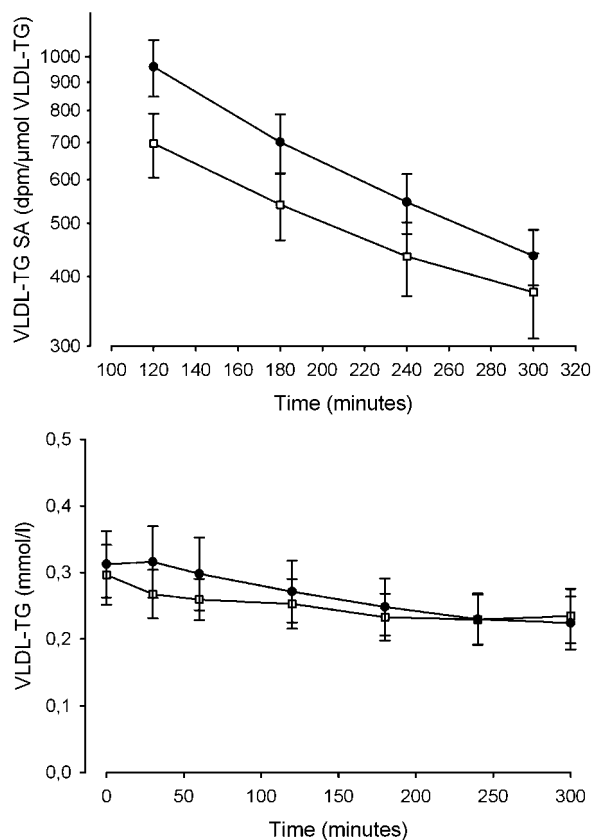


Fig. 1. Top panel: VLDL-triglyceride (TG) specific activity (SA) versus time for men (open squares) and women (closed circles). Bottom panel: TG levels through the study period. ANOVA for repeated measurements showed no significant effect of sex or time on VLDL-TG concentration.

TABLE 2. Metabolic parameters

Parameter	Men (n = 13)	Women (n = 12)	P
Mean p-VLDL-TG (mmol/l)	0.26 ± 0.03	0.27 ± 0.04	NS
Mean p-total TG (mmol/l)	0.77 ± 0.07	0.87 ± 0.09	NS
VLDL-TG FCR (pools/h)	0.23 ± 0.02	0.26 ± 0.01	NS
VLDL-TG production (μmol/min)	3.20 ± 0.32	2.82 ± 0.42	NS
VLDL-TG secretion (μmol/l plasma/min)	0.9 ± 0.1	1.1 ± 0.2	NS
VLDL-TG clearance (ml/min)	14.2 (7.2–23.6)	10.0 (6.8–13.7)	NS
p-Palmitate (μmol/l)	118 ± 11	117 ± 9	NS
Palmitate flux (μmol/min)	128.5 (91–226)	120.5 (78–250)	NS
Lipid oxidation (kcal/24 h)	693 ± 55	450 ± 74	<0.05
Carbohydrate oxidation (kcal/24 h)	683 ± 59	628 ± 45	NS
Protein oxidation (kcal/24 h)	466 ± 50	360 ± 40	NS

FCR, fractional catabolic rate; p, plasma; TG, triglyceride. Data are means ± SEM or median and (range).

include both the nonstepped and the stepwise (forward) regression analyses. Using the stepwise selection method, we found that the best multivariate model to predict VLDL-TG production included log-insulin (slope, 1.06; $P = 0.02$) and REE (slope, 0.0021; $P = 0.02$). The adjusted r^2 for this model was 0.32. Forcing sex or indices of body composition into this model did not improve the overall r^2 . However, if data from the outlier in Fig. 2 (lower panel) were excluded, sex emerged as a significant independent determinant of VLDL-TG production.

Figure 3 shows the relationship between VLDL-FCR and VLDL-TG pool size. A significant inverse correlation was found, indicating a progressively slow relative turnover of the VLDL-TG pool with increasing VLDL-TG pool size.

DISCUSSION

This study was undertaken to determine the factors that affect VLDL-TG production. We measured VLDL-TG production using a recently validated method based on the plasma disappearance rate of a bolus infusion of ex vivo labeled VLDL-TG particles (12). Our results show that total VLDL-TG production (μmol/min) is similar in men and women. Furthermore, we found REE and plasma insulin, but not palmitate turnover or palmitate concentration, to be independent, significant predictors of VLDL-TG production.

Whereas an association between fasting plasma insulin and VLDL-TG production has been reported previously (9), it is in our opinion a novel observation that REE determines hepatic VLDL-TG production. Moreover, the relationship improved when men and women were examined separately, indicating a modification by sex of VLDL production independent of REE. VLDL-TG production increased similarly as a function of REE in men and women, whereas the regression line intercepts differed significantly. As a result, VLDL-TG production was ~40% greater in women than in men when compared at

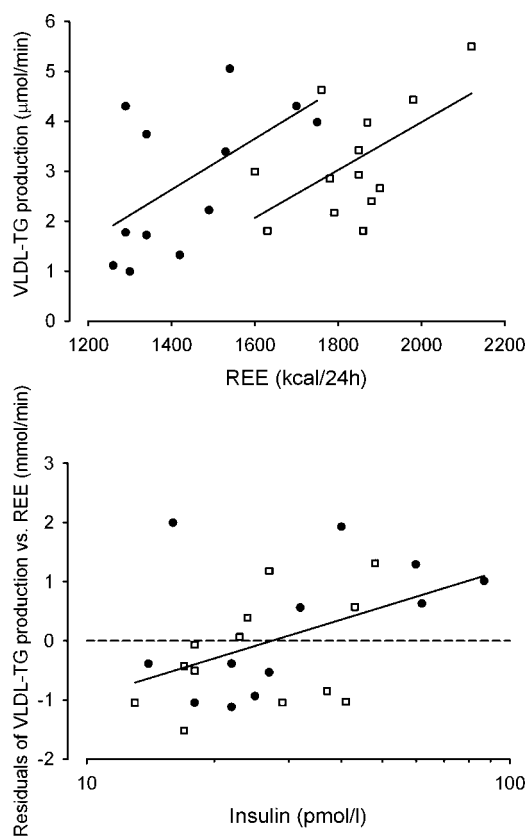


Fig. 2. Top panel: Relationship between VLDL-TG production and resting energy expenditure (REE) in women (closed circles) and men (open squares). Notice that sex-specific univariate correlations between REE and VLDL-TG production were greater (men, $r = 0.57$, $P < 0.05$; women, $r = 0.58$, $P < 0.05$) than that for the entire group ($r = 0.45$, $P = 0.02$). The intercept of the relationship between REE and VLDL-TG production was significantly different between men and women ($P < 0.05$). Bottom panel: Residuals of VLDL-TG production versus REE plotted against fasting insulin levels for women (closed circles) and men (open squares). Pearson's correlation for the combined group was $r = 0.46$, $P = 0.02$.

similar REE and relative fuel (glucose and lipid) utilization levels. Because lipid oxidation (indirect calorimetry) is greater in men, this relative excess VLDL-TG production in women appears not to be oxidized but rather directed toward nonoxidative disposal (e.g., storage in adipose tissue). However, because lipoprotein lipase is also present in lean tissues, it is possible that VLDL-TG contributes relatively more to total lipid uptake in lean tissues in women than in men. In fact, the presence of an independent relationship of VLDL-TG production with REE but not with FFA turnover or FFA concentration suggests that VLDL-TG particles may be involved in a direct way in substrate (FFA) delivery to energy-consuming tissues (e.g., skeletal muscle and heart) in the postabsorptive state. Although this suggestion is based on a statistical association, there are indications in the literature that the reverse is not the case. Thus, REE is not increased when VLDL-TG is increased by infusion of TG-rich intralipid emulsions (18), suggesting that prevailing energy needs may play a greater role in VLDL-TG production than was previously antici-

TABLE 3. Univariate correlations

Variable	VLDL Production ($\mu\text{mol}/\text{min}$)		FCR (pools/h)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
p-Palmitate ($\mu\text{mol}/\text{l}$)	-0.2672	0.197	0.2192	0.292
Palmitate flux ($\mu\text{mol}/\text{min}$)	0.0916	0.67	0.3059	0.146
REE (kcal/24 h)	0.4545	0.022	-0.1701	0.416
Intra-abdominal fat (cm^2)	0.2744	0.229	0.4105	0.065
Fat mass (g)	0.2463	0.235	0.4143	0.039
p-Insulin (pmol/l)	0.4593	0.021	-0.1748	0.403
VLDL production ($\mu\text{mol}/\text{min}$)	—	—	-0.1432	0.495
VLDL pool (μmol)	0.7565	<0.0001	-0.5857	0.002
Sex (woman)	-0.1487	0.478	0.2605	0.209

Univariate correlations with $P > 0.5$ are not shown.

pated. As opposed to previous belief, however, FFA turnover or concentration was not significantly associated with VLDL-TG production (19) in this study.

Upper body obesity, especially when associated with the visceral accumulation of adipose tissue, is associated with an increased release of FFAs into the portal vein, which may promote hepatic TG formation (6, 20). Accordingly, studies have shown that upper body obese individuals produce more VLDL-TG than lean subjects (21, 22). However, when circulating FFAs are increased experimentally, conflicting results appear; some have reported increased VLDL-TG production after intravenous infusion of FFAs (intralipid) and heparin (11), but this effect cannot be reproduced when FFA levels are increased by exercise (23). Conversely, administration of acipimox, an inhibitor of adipose tissue lipolysis, results in decreased VLDL-TG production (24). We found no significant relationship between VLDL-TG production and palmitate flux or palmitate concentrations. Nor did we find any independent relationship of measures of body size or body composition with VLDL-TG production. However, as participants were recruited to cover a wide range of body weight and composition, no direct stratification (e.g., lean vs. obese) was attempted. Our inability to detect a relationship between palmitate levels and VLDL-TG production is partly supported by other studies, which failed to detect a correlation between FFA and VLDL-TG kinetics (9, 25, 26). In addition, our results may reflect the low variability in FFA turnover associated with the postabsorptive state, as opposed to the large-scale manipulation of FFAs from infusion of intralipid, catecholamines, or insulin.

TABLE 4. Multivariate, nonstepped, regression analysis

Variable	Parameter Estimate	Standard Error	<i>P</i>
Intercept	-7.29	3.39	0.044
Fat mass (g)	-0.000017	0.0000351	0.63
Log insulin (pmol/l)	0.89	0.54	0.114
REE (kcal/24 h)	0.0043	0.0019	0.036
Sex (woman)	1.34	1.08	0.227

Dependent variable: VLDL-TG production ($\mu\text{mol}/\text{min}$).

TABLE 5. Multivariate, stepwise forward, regression analysis

Variable	Parameter Estimate	Standard Error	<i>P</i>
Intercept	-3.95	1.92	0.05
Log insulin (pmol/l)	1.06	0.43	0.02
REE (kcal/24 h)	0.0021	0.0009	0.02

Dependent variable: VLDL-TG production ($\mu\text{mol}/\text{min}$).

The effect of insulin on VLDL-TG production appears to depend on the nature of the insulin exposure. Acute hyperinsulinemia (hours) decreases VLDL-TG production (10, 11), whereas prolonged hyperinsulinemia (days) increases VLDL-TG production (8). Both animal (27, 28) and human (29) studies indicate insulin resistance as a likely cause of VLDL-TG overproduction, and our finding of a positive correlation between fasting insulin level and VLDL-TG production appears to be in agreement with this. Our data do not allow further speculation on the exact action of insulin on VLDL-TG production, because no controlled manipulation of insulin concentrations was performed. However, because insulin levels generally are greater in obese insulin-resistant subjects than in lean subjects, one might speculate whether the previously reported effect of obesity on VLDL-TG production (21) could be indirect via hyperinsulinemia, possibly combined with insulin-resistant adipose tissue lipolysis.

Previous studies of VLDL-TG production have been limited by few subjects investigated (8, 24, 29–32) or study designs restricted to only one sex (8, 24, 30–33). Thus, few studies have examined whether sex-specific differences in VLDL-TG production exist. Our findings are in agreement with some studies (9, 10, 34) in which VLDL-TG production appeared not to differ between the sexes. Recently, however, studies have reported independent effects of sex on VLDL-TG and FFA kinetics (21, 35). Normal weight women were found to clear VLDL-TG more rapidly than men, resulting in a faster turnover rate of their TG pool (21). In theory, this higher turnover may reflect a smaller

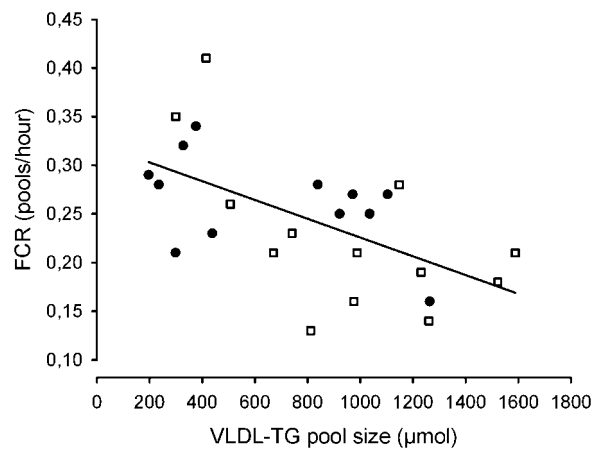


Fig. 3. Relationship between fractional catabolic rate (FCR; pools/hour) and VLDL-TG pool size (μmol). Pearson's correlation was $r = -0.59$, $P < 0.01$. Data from men (open squares) and women (closed circles) were combined.

TG pool size, a more efficient lipid oxidation, or, alternatively, an increased channeling into nonoxidative disposal (i.e., storage in adipose tissue). Contrary to the findings of Mittendorfer, Patterson, and Klein (21), however, we did not find men and women to differ significantly in terms of both absolute and relative (FCR) VLDL-TG turnover. Indeed, men tended to have higher basal VLDL-TG production than women, although this did not reach the level of significance.

The observation of a close correlation between VLDL-TG pool size and FCR is supported by a recent article reviewing some methodological problems concerning kinetic studies of VLDL-TG, particularly the interrelationship between pool size and FCR (2). The authors concluded that the differences of FCR and associated parameters (production, secretion into plasma, and clearance) between various studies may result from varying TG pool sizes. The same relationship between total VLDL-TG pool size and FCR was found in the present study: subjects with small VLDL-TG pool sizes had a high relative turnover of that pool, and subjects with large VLDL-TG pools had a slower turnover. Because of the descriptive nature of this study, our findings are of course strictly observational and offer no suggestions regarding which factors determine the set point for VLDL-TG pool size and VLDL-TG concentration.

In conclusion, we offer a novel approach to understanding VLDL-TG kinetics in the overnight-fasted state. In our model, VLDL-TG production is similar in women and men but also independently determined by REE and plasma insulin levels. Therefore, these factors must be taken into account when evaluating VLDL-TG kinetics, especially when groups of both men and women are examined. Finally, we propose a proportionally greater role for VLDL-TG with respect to lipid fuel delivery to energy-consuming tissues in the overnight-fasted state. ■

This work was supported by grants from the Danish Medical Research Council, the Novo Nordic Foundation, and the Danish Diabetes Foundation (to S.N.). The authors acknowledge the excellent technical assistance of Ms. Elin Carstensen, Ms. Lene Ring, Ms. Lone Svendsen, and Ms. Susanne Sørensen.

REFERENCES

- Hokanson, J. E., and M. A. Austin. 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk*. **3**: 213–219.
- Magkos, F., and L. S. Sidossis. 2004. Measuring very low density lipoprotein-triglyceride kinetics in man in vivo: how different the various methods really are. *Curr. Opin. Clin. Nutr. Metab. Care*. **7**: 547–555.
- Lam, T. K., A. Carpentier, G. F. Lewis, G. van de Werve, I. G. Fantus, and A. Giacca. 2003. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. *Am. J. Physiol. Endocrinol. Metab.* **284**: E863–E873.
- Lewis, G. F. 1997. Fatty acid regulation of very low density lipoprotein production. *Curr. Opin. Lipidol.* **8**: 146–153.
- Nielsen, S., Z. Guo, J. B. Albu, S. Klein, P. C. O'Brien, and M. D. Jensen. 2003. Energy expenditure, sex, and endogenous fuel availability in humans. *J. Clin. Invest.* **111**: 981–988.
- Jensen, M. D., M. W. Haymond, R. A. Rizza, P. E. Cryer, and J. M. Miles. 1989. Influence of body fat distribution on free fatty acid metabolism in obesity. *J. Clin. Invest.* **83**: 1168–1173.
- Jensen, M. D. 2003. Fate of fatty acids at rest and during exercise: regulatory mechanisms. *Acta Physiol. Scand.* **178**: 385–390.
- Aarsland, A., D. Chinkes, and R. R. Wolfe. 1996. Contributions of de novo synthesis of fatty acids to total VLDL-triglyceride secretion during prolonged hyperglycemia/hyperinsulinemia in normal man. *J. Clin. Invest.* **98**: 2008–2017.
- Carpentier, A., B. W. Patterson, N. Leung, and G. F. Lewis. 2002. Sensitivity to acute insulin-mediated suppression of plasma free fatty acids is not a determinant of fasting VLDL triglyceride secretion in healthy humans. *Diabetes*. **51**: 1867–1875.
- Lewis, G. F., K. D. Uffelman, L. W. Szeto, and G. Steiner. 1993. Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes*. **42**: 833–842.
- Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95**: 158–166.
- Gormsen, L. C., M. D. Jensen, and S. Nielsen. 2006. Measuring VLDL-triglyceride turnover in humans using ex vivo-prepared VLDL tracer. *J. Lipid Res.* **47**: 99–106.
- Jensen, M. D., J. A. Kanaley, J. E. Reed, and P. F. Sheedy. 1995. Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. *Am. J. Clin. Nutr.* **61**: 274–278.
- Ferrannini, E. 1988. The theoretical bases of indirect calorimetry: a review. *Metabolism*. **37**: 287–301.
- Guo, Z., S. Nielsen, B. Burguera, and M. D. Jensen. 1997. Free fatty acid turnover measured using ultralow doses of [U - ^{13}C]palmitate. *J. Lipid Res.* **38**: 1888–1895.
- Reaven, G. M., D. B. Hill, R. C. Gross, and J. W. Farquhar. 1965. Kinetics of triglyceride turnover of very low density lipoproteins of human plasma. *J. Clin. Invest.* **44**: 1826–1833.
- Boer, P. 1984. Estimated lean body mass as an index for normalization of body fluid volumes in humans. *Am. J. Physiol. Endocrinol. Metab.* **247**: F632–F636.
- Boden, G., and G. I. Shulman. 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur. J. Clin. Invest.* **32**(Suppl. 3): 14–23.
- Lewis, G. F. 1997. Fatty acid regulation of very low density lipoprotein production. *Curr. Opin. Lipidol.* **8**: 146–153.
- Nielsen, S., Z. Guo, C. M. Johnson, D. D. Hensrud, and M. D. Jensen. 2004. Splanchnic lipolysis in human obesity. *J. Clin. Invest.* **113**: 1582–1588.
- Mittendorfer, B., B. W. Patterson, and S. Klein. 2003. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. *Am. J. Clin. Nutr.* **77**: 573–579.
- Mittendorfer, B., B. W. Patterson, and S. Klein. 2003. Effect of weight loss on VLDL-triglyceride and apoB-100 kinetics in women with abdominal obesity. *Am. J. Physiol. Endocrinol. Metab.* **284**: E549–E556.
- Magkos, F., D. C. Wright, B. W. Patterson, B. S. Mohammed, and B. Mittendorfer. 2006. Lipid metabolism response to a prolonged bout of endurance exercise in healthy young men. *Am. J. Physiol. Endocrinol. Metab.* **290**: E355–E362.
- Wang, W., A. Basinger, R. A. Neese, B. Shane, S. A. Myong, M. Christiansen, and M. K. Hellerstein. 2001. Effect of nicotinic acid administration on hepatic very low density lipoprotein-triglyceride production. *Am. J. Physiol. Endocrinol. Metab.* **280**: E540–E547.
- Riches, F. M., G. F. Watts, R. P. Naoumova, J. M. Kelly, K. D. Croft, and G. R. Thompson. 1998. Hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 studied with a stable isotope technique in men with visceral obesity. *Int. J. Obes. Relat. Metab. Disord.* **22**: 414–423.
- Cummings, M. H., G. F. Watts, C. Pal, M. Umpleby, T. R. Hennessy, R. Naoumova, and P. H. Sonksen. 1995. Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in obesity: a stable isotope study. *Clin. Sci.* **88**: 225–233.
- Koopmans, S. J., R. S. Kushwaha, and R. A. DeFronzo. 1999. Chronic physiologic hyperinsulinemia impairs suppression of plasma free fatty acids and increases de novo lipogenesis but does not cause dyslipidemia in conscious normal rats. *Metabolism*. **48**: 330–337.
- Taghibiglou, C., A. Carpentier, S. C. Van Iderstine, B. Chen, D. Rudy, A. Aiton, G. F. Lewis, and K. Adeli. 2000. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced

intracellular apoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *J. Biol. Chem.* **275**: 8416–8425.

29. Carpentier, A., B. W. Patterson, K. D. Uffelman, A. Giacca, M. Vranic, M. S. Cattral, and G. F. Lewis. 2001. The effect of systemic versus portal insulin delivery in pancreas transplantation on insulin action and VLDL metabolism. *Diabetes*. **50**: 1402–1413.
30. Lemieux, S., B. W. Patterson, A. Carpentier, G. F. Lewis, and G. Steiner. 1999. A stable isotope method using a [(2)H(5)]glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans. *J. Lipid Res.* **40**: 2111–2117.
31. Melish, J., N. A. Le, H. Ginsberg, D. Steinberg, and W. V. Brown. 1980. Dissociation of apoprotein B and triglyceride production in very-low-density lipoproteins. *Am. J. Physiol. Endocrinol. Metab.* **239**: E354–E362.
32. Kissebah, A. H., P. W. Adams, and V. Wynn. 1974. Plasma free fatty acid and triglyceride transport kinetics in man. *Clin. Sci. Mol. Med.* **47**: 259–278.
33. Zech, L. A., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides. Evidence for a slow production pathway and results for normolipidemic subjects. *J. Clin. Invest.* **63**: 1262–1273.
34. Nikkila, E. A., and M. Kekki. 1971. Polymorphism of plasma triglyceride kinetics in normal human adult subjects. *Acta Med. Scand.* **190**: 49–59.
35. Mittendorfer, B., B. W. Patterson, S. Klein, and L. S. Sidossis. 2003. VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity. *Am. J. Physiol. Endocrinol. Metab.* **284**: E708–E715.