Gender Differences in VLDL₁ and VLDL₂ Triglyceride Kinetics and Fatty Acid Kinetics in Obese Postmenopausal Women and Obese Men

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Context: High plasma triglycerides (TG) have been shown to be independent and better predictors of cardiovascular disease than low-density lipoprotein (LDL) cholesterol in women. This may be due to gender differences in very-low-density lipoprotein 1 (VLDL₁)- and VLDL₂-TG and fatty acid kinetics.

Objective: Our objective was to investigate whether there are differences in VLDL₁- and VLDL₂-TG and fatty acid kinetics in obese men and postmenopausal women, a high risk group for cardiovascular disease.

Research Design and Methods: Stable isotopes techniques were used to measure fasting palmitate rate of appearance, metabolic clearance rate, oxidation rate, and nonoxidative disposal rate, VLDL₁-TG and VLDL₂-TG fractional catabolic rate (FCR) and production rate (PR). Whole-body fat distribution was measured by magnetic resonance imaging.

Participants: Participants included 10 postmenopausal obese women and eight obese men matched for age, body mass index, and fasting plasma TG.

Results: The women had lower visceral fat and higher sc fat than the men (P < 0.001 and P < 0.002). Palmitate rate of appearance, metabolic clearance rate, nonoxidative disposal rate, and oxidation rate corrected for resting energy expenditure were greater in the women than the men (all P < 0.03). VLDL₂-TG PR corrected for fat-free mass was higher in the women (P < 0.001). VLDL₂-TG and VLDL₂-cholesterol pools were higher in the women (P < 0.001 and P < 0.008). VLDL₁-TG FCR and PR and VLDL₂-TG FCR were not different between genders.

Conclusion: Fatty acid and VLDL₂-TG flux is higher in postmenopausal obese women than in obese men matched for fasting plasma TG levels. (J Clin Endocrinol Metab 97: 2475–2481, 2012)
H High plasma triglycerides (TG) are independent and better predictors of cardiovascular disease (CVD) than low-density lipoprotein (LDL) cholesterol in women (1), suggesting that treatment of hypertriglyceridemia may be more important in women. This finding may be related to differences in lipid metabolism between genders. Women are at a lower risk of cardiovascular disease than men until they are postmenopausal, when the risk is similar (2). The reason for this is not clear but may be related to the increase in fat in postmenopausal women rather than the loss of ovarian hormones (3). Obesity is one of the major risk factors for the development of CVD in both men and women (4).

In young lean subjects, it has been shown that very-low-density lipoprotein (VLDL)-TG production by the liver is higher in women than men, but because clearance was also much higher in women, this resulted in lower plasma TG in women than men (5). In young obese men, VLDL-TG production was higher than in young obese women, and clearance was similar in the two groups, also resulting in lower plasma TG in women than men (6). Although these studies clearly indicate there are major differences in lipoprotein metabolism in men and women, if we are to understand why plasma TG is a better predictor of CVD in women, VLDL kinetics studies need to be undertaken in men and women matched for plasma TG concentration.

VLDL secreted by the liver can be divided into the large TG-rich VLDL₁ and the smaller more dense TG-poor VLDL₂. There is evidence that these two VLDL species are independently regulated (7). The first step in VLDL assembly is the formation of a partially assembled primordial particle (pre-VLDL). This is formed when apolipoprotein B100 is cotranslationally lipidated in the endoplasmic reticulum by (pre-VLDL). This is formed when apolipoprotein B100 is independently regulated (7). The first step in VLDL assembly is maintained and degraded or further lipidated to form VLDL₂. There is evidence that these two VLDL species are matched for adiposity (13). Although FFA concentrations have been reported to be higher in women (14), the difference is small, and many studies find no difference in FFA concentrations (15). This suggests that FFA clearance from the circulation must also be higher in women than men. The increased delivery of FFA to liver may lead to increased VLDL-TG production in women.

Using stable isotope techniques, this study has investigated in postmenopausal obese women and men matched for age, body mass index (BMI), and fasting plasma TG whether there are differences in VLDL₁-TG and VLDL₂-TG production and clearance rate, FFA production, and oxidation rate.

Subjects and Methods

Study design

This study was approved by the East Kent Ethics Committee and University of Surrey Ethics Committee. Written informed consent was provided by all subjects before inclusion in the study. Subjects were excluded if they were diagnosed with diabetes or suffering from either cardiovascular or endocrine disease or hepatic or renal disorders. Exclusion criteria also included the prescribed use of any medications known to alter body weight or appetite, β-blockers, fibrates, and metformin.

Eight obese men (60 ± 3 yr, BMI = 32 ± 1 kg/m²) (mean ± SEM) and 10 postmenopausal obese women (58 ± 2 yr, BMI = 33 ± 1 kg/m²) with fasting plasma TG no higher than 1.7 mmol/liter participated in the study. Measurement of 1) resting energy expenditure (REE) and the acetate correction factor and 2) palmitate and TG kinetics were measured on two separate study days. Body composition was measured by magnetic resonance imaging. Subjects were asked not to undertake any vigorous exercise or drink any alcohol for 48 h before both study days. Subjects were provided with a standardized low-fat, low-fiber meal the evening before both study days and then fasted overnight.

Study 1

Twenty-four-hour urine samples were collected the previous day for the measurement of nitrogen excretion. REE and fat and carbohydrate oxidation were measured using a ventilated hood open-circuit indirect calorimeter (gas exchange monitor; GEM-Nutrition Ltd., Cheshire, UK). Subjects were allowed to rest for 30 min in a quiet room and completed three practice measurements to avoid a training effect. A 40-min measurement of REE was then made. This was followed by a priming dose of [1-13C]sodium bicarbonate (0.085 mg/kg) and a 2-h infusion of [1,2-13C]acetate (0.08 μmol/kg·min) via an indwelling venous cannula. Measurements of 13CO₂ production rate (PR) were made to correct palmitate oxidation for the loss of label in the tricarboxylic acid cycle (16). Breath samples were collected at baseline and then every 10 min from 90–120 min for the measurement of 13CO₂. CO₂ PR was measured with the gas exchange monitor.
Study 2

Study 2 took place 3 d after study 1. A baseline blood sample was taken to measure the baseline enrichment and concentration of plasma palmitate and the glycerol enrichment of VLDL1- and VLDL2-TG. A priming dose of \([1,1,2-13C]\)acetate (0.5 mmol) was administered to measure VLDL1- and VLDL2-TG metabolism. Blood samples were taken at intervals for 7 h to measure VLDL1- and VLDL2-TG metabolism. Blood samples were taken at intervals for 7 h to measure the palmitate production and oxidation rate. Blood and breath samples were taken every 10 min from 90–120 min for the measurement of the enrichment and concentration of plasma palmitate and breath \({}^{13}\text{CO}_2\). CO2 PR was measured with the gas exchange monitor.

Body composition

On a separate day, adipose tissue content, intrahepatocellular lipid (IHCL), and intramyocellular lipid (IMCL) were measured at the Medical Research Council Clinical Sciences Centre Ham- mersmith Hospital. Subjects fasted for 6 h before the scans. Whole-body magnetic resonance imaging of body fat content and IHCL and IMCL levels were acquired on an Intera 1.5-T Achieva multinuclear system (Philips Medical Systems, Best, The Netherlands) as previously reported (17). IMCL spectra were obtained from the tibialis muscle group, which contains predominantly glycolytic fibers and soleus muscle. Total and regional adipose tissue volumes were measured after imaging data were analyzed using the SliceOmatic image analysis program (Tomovision, Montreal, Quebec, Canada). All spectra were analyzed in the time domain using the AMARES algorithm included in the MRUI software package (18). IMCL was expressed as a ratio to the muscle creatine signal. IHCL was expressed as a ratio to liver water content.

Laboratory methods

VLDL1- (Svedberg flotation rate 60–400) and VLDL2- (Svedberg flotation rate 20–60) were separated by sequential ultracentrifugation as described previously (19). VLDL1- and VLDL2-TG were extracted with chloroform-methanol (2:1), isolated by thin layer chromatography, and hydrolyzed in a solution of 2% HCl in methanol, and the liberated glycerol was purified by ion exchange chromatography and derivatized to form the triacetate derivative (20). Isotopic enrichment was measured by positive chemical ionization gas chromatography mass spectrometry (GCMS) (Agilent 5973 network MSD) monitoring the ions 159 and 164. FFA were extracted from plasma and derivatized to their methyl esters. The isotopic enrichment of the palmitic acid methyl ester was measured by GCMS monitoring the ions 270 and 286. CO2 enrichment was measured by isotope ratio MS (Delta XP, coupled with a Gas Bench II; ThermoElectron, Bremen, Germany). Plasma palmitate concentration was measured by GCMS after addition of an internal standard, heptadecanoic acid.

Urine nitrogen concentration was measured with a LECO model FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI). Enzymatic assays were used to measure plasma FFA, total cholesterol, TG, 3-hydroxybutyrate, VLDL1- and VLDL2-TG (ABX; Chicksands, Shefford, Bedfordshire, UK), and glycerol (ABX; Chicksands, Shefford, Bedfordshire, UK). High-density lipoprotein (HDL)-cholesterol was measured enzymatically after precipitation of apolipoprotein B-containing proteins with dextran sulfate/magnesium chloride using a stable liquid reagent-immunoinhibition method (HDL-C L-Type, Alpha Labs, Eastleigh, Hampshire, UK). Insulin, adiponectin, and leptin were measured by immunoassay using commercially available kits (Millipore Corp., Billerica, MA).

Data analysis

Tracer enrichment of palmitate, glycerol, and CO2 was expressed as tracer-to-tracer ratio (TTR) corrected for baseline enrichment. Palmitate rate of appearance (Ra) was calculated as infusion rate of tracer/ TTR. Palmitate Ra was assumed to equal the rate of disappearance (Rd). Palmitate metabolic clearance rate (MCR) was calculated by dividing palmitate Rd by palmitate concentration. The PR of \(^{13}\text{CO}_2\) from the infused palmitate tracer was calculated as \(\text{CO}_2\ TTR \times \text{VCO}_2/k \cdot \text{Ac}_\text{recovery}\) where \(\text{VCO}_2\) is carbon dioxide production (liters per minute), \(k\) is the volume of 1 mol \(\text{CO}_2\) (22.4 liters), and \(\text{Ac}_\text{recovery}\) is the fractional 13C label recovery in breath CO2, observed after the infusion of labeled acetate (16) and calculated as \(\text{CO}_2\ TTR \times \text{VCO}_2/k \cdot \text{Ac}_\text{recovery}\). Plasma palmitate oxidation was calculated as palmitate Rd \(\times\) \(^{13}\text{CO}_2\ PR/F \times 16\) where F is the palmitate enrichment rate and 16 is the number of carbon atoms in palmitate. Plasma nonoxidative disposal was calculated as the difference between palmitate Rd and palmitate oxidation rate. Palmitate kinetics was expressed in terms of both REE and fat-free mass (FFM). The latter was calculated as the difference between body weight and total body fat measured by magnetic resonance imaging. VLDL1- and VLDL2-TG fractional catabolic rate (FCR) was calculated using a compartment model of VLDL1-TG and VLDL2-TG kinetics (8) using the SAAM II program (SAAM Institute, Seattle, WA). The model represents the kinetics of the TTR profiles, which change as labeled glycerol is removed from plasma and incorporated into the TG fractions. The model assumes steady state of native (unlabeled) glycerol throughout the experimental period, i.e. a constant appearance, disappearance, and incorporation of native glycerol into the TG fractions. The incorporation of glycerol into VLDL by the liver is subject to a delay, and two delay pathways were represented (21). The model included a compartment for VLDL1-TG and a compartment for VLDL2-TG with an input into both compartments from the glycerol precursor pool, a loss from each compartment, and a transfer from the VLDL1-TG compartment to the VLDL2-TG compartment. The direct input into VLDL2-TG is termed VLDL1-TG hepatic PR, whereas the transfer from VLDL1-TG into VLDL2-TG is termed VLDL1- to VLDL2-TG transfer rate. Figure 1 shows a typical curve fit. VLDL1- and VLDL2-TG pools were calculated from VLDL1- and VLDL2-TG concentration and plasma volume, which was determined by the method of Pearson et al. (22). Total VLDL-TG PR was calculated as the sum of VLDL1-TG PR and VLDL2-TG hepatic PR. VLDL kinetics are expressed in terms of FFM.

Carbohydrate and fat oxidation were calculated from the measurements of oxygen consumption and carbon dioxide production corrected for protein oxidation, calculated from urinary
nitrogen excretion, as described by Frayn (23). Fasting insulin sensitivity was assessed by homeostatic model assessment of insulin resistance (HOMA-IR) (24).

Statistical analysis

The results are presented as means ± SEM. Differences between groups were analyzed by Student’s t test using SPSS (version 16). Nonparametric data were logarithmically transformed before analysis. Associations between variables of interest were performed using correlation analysis.

Results

Body composition and energy expenditure

Body weight, FFM, and REE were higher in the men (P = 0.001, P < 0.001, and P < 0.001 respectively). Total fat mass was not significantly different between genders, but sc fat mass and abdominal sc fat were higher in the women, (P < 0.002 and P < 0.05), whereas visceral fat was lower (P < 0.001). BMI, waist circumference, IHCL, and IMCL were not different between genders. Total fat and carbohydrate oxidation also did not differ between genders (Table 1).

Hormones, lipids, and insulin sensitivity

Fasting serum insulin, plasma glucose, and HOMA-IR were not different between genders. Leptin was higher in the women (P < 0.001), but adiponectin was not different. There was no difference in plasma TG, total cholesterol, LDL, or HDL cholesterol. The concentration of 3-Hydroxybutyrate was not different in men and women (Table 2).

Palmitate kinetics

Although FFA concentrations were higher in the women, this was not statistically significant. Glycerol concentrations were markedly higher (P < 0.001) compared with the men. Palmitate flux, MCR, and oxidation rate were significantly higher in the women than men expressed as FFM (P < 0.004, P < 0.005, and P < 0.002) or REE (P < 0.02, P < 0.03, and P < 0.03). The higher palmitate oxidation rate in the women only partially accounted for the higher palmitate flux; thus, there was also a higher palmitate nonoxidative disposal in the women compared with the men expressed as FFM or REE (P < 0.01 and P < 0.03) (Table 3).

VLDL kinetics

Total VLDL-TG PR was 0.38 ± 0.05 in the women and 0.30 ± 0.04 in the men (not significantly different between genders). The VLDL1-TG pool and VLDL1-TG FCR and PR were also not significantly different between genders. Plasma concentrations of VLDL1-TG were not different in women (0.70 ± 0.08 mmol/liter) and men (0.74 ± 0.09 mmol/liter).

| Table 1. Body composition and energy expenditure (mean ± SEM) |
| --- | --- | --- |
| | Men | Women | P value |
| Age (yr) | 60 ± 3 | 58 ± 2 | NS |
| Body weight (kg) | 104.2 ± 4.7 | 85.4 ± 2.2 | 0.001 |
| BMI | 32.0 ± 0.3 | 32.6 ± 0.6 | NS |
| Waist (cm) | 111.1 ± 1.9 | 104.8 ± 3.3 | NS |
| Fat mass (kg) | 35.8 ± 2.4 | 41.3 ± 1.6 | NS |
| FFM (kg) | 68.6 ± 2.5 | 41.1 ± 1.2 | <0.001 |
| Visceral fat (kg) | 5.7 ± 0.5 | 3.5 ± 0.3 | <0.001 |
| sc fat (kg) | 25.2 ± 2.5 | 34.7 ± 1.6 | <0.002 |
| Abdominal sc fat (kg) | 7.59 ± 1.00 | 10.18 ± 0.72 | <0.05 |
| IHCL (%) | 15.5 ± 4.7 | 13.20 ± 7.0 | NS |
| Soleus IMCL | 17.5 ± 2.0 | 14.6 ± 2.2 | NS |
| Tibialis IMCL | 9.4 ± 0.9 | 7.8 ± 0.7 | NS |
| REE (kcal/d) | 1912 ± 49 | 1420 ± 49 | <0.001 |
| Carbohydrate oxidation (mg/kg FFM min) | 2.75 ± 0.22 | 2.91 ± 0.22 | NS |
| Fat oxidation (mg/kg FFM min) | 0.66 ± 0.10 | 0.70 ± 0.11 | NS |

| Table 2. Hormones, lipids, and insulin sensitivity |
| --- | --- | --- |
| | Men | Women | P value |
| Insulin (pmol/liter) | 112.9 ± 9.7 | 97.0 ± 10.0 | NS |
| Glucose (mmol/liter) | 5.5 ± 0.1 | 5.5 ± 0.1 | NS |
| HOMA-IR | 2.1 ± 0.3 | 1.8 ± 0.2 | NS |
| TG (mmol/liter) | 1.17 ± 0.10 | 1.21 ± 0.07 | NS |
| Cholesterol (mmol/liter) | 5.2 ± 0.3 | 5.6 ± 0.2 | NS |
| LDL cholesterol (mmol/liter) | 3.4 ± 0.3 | 3.7 ± 0.2 | NS |
| HDL cholesterol (mmol/liter) | 1.24 ± 0.10 | 1.36 ± 0.06 | NS |
| 3-Hydroxybutyrate (mmol/liter) | 0.20 ± 0.06 | 0.13 ± 0.05 | NS |
| Adiponectin (µg/ml) | 8.9 ± 1.6 | 12.1 ± 1.2 | NS |
| Leptin ng/ml | 8.8 ± 1.1 | 21.2 ± 2.2 | <0.001 |

NS, Not significant.
mmol/liter), but VLDL₂-TG concentrations were significantly higher in women than men (0.21 ± 0.02 vs. 0.10 ± 0.01; P < 0.001). The VLDL₂-TG pool and VLDL₂-TG PR were significantly higher in the women (P < 0.001, P < 0.001). The latter was due to both a higher hepatic production of VLDL₂-TG in the women than the men (P < 0.007) as well as a higher VLDL₁⁻ to VLDL₂-TG transfer rate in the women than the men (P < 0.005). VLDL₂-TG FCR was not significantly different between genders. The VLDL₁ cholesterol pool was not different between genders, but the VLDL₂ cholesterol pool was higher in the women (P < 0.008). There was no correlation between nonoxidative palmitate Rd and VLDL₁⁻, VLDL₂⁻, or total VLDL-TG PR (Table 3).

**Discussion**

After the menopause, women have the same risk for CVD as men. However, unlike men, in women, high plasma TG are independent and better predictors of CVD than LDL cholesterol (1). In this study, we investigated whether there were any differences in TG and FFA kinetics between obese men and obese premenopausal women matched for BMI, age, and fasting plasma TG levels. Palmitate was selected to assess FFA kinetics because it is one of the major FFA. The study showed that obese postmenopausal women, with the same fasting plasma TG level as men, have a higher fatty acid flux relative to both FFM and REE than men and also higher VLDL₂-TG PR due to both a higher hepatic PR and a higher VLDL₁⁻ to VLDL₂-TG transfer rate. Plasma fatty acid oxidation was also higher in women than men, but it did not fully account for the higher fatty acid flux so that nonoxidative fatty acid Rd was also elevated.

FFA flux relative to FFM has previously been shown to be higher in postmenopausal women than men, although the subjects were not age matched (25). This is the first study to show this in obese age-matched postmenopausal women and men. Nielsen et al. (15) showed in lean and obese premenopausal women that FFA flux relative to REE was 40% higher than in men of a similar BMI and age. The current study also found FFA flux relative to REE was 40% higher in postmenopausal women than men, suggesting that postmenopausal status does not affect the gender difference in fatty acid flux. Although plasma FFA oxidation was higher in women than men, total lipid oxidation, measured by indirect calorimetry, was not different between genders, which suggests that men may oxidize more nonplasma lipids. Koutsari et al. (26) measured total lipid oxidation by indirect calorimetry and subtracted this from total FFA flux to obtain a measure of nonoxidative FFA disposal in young men and women and also concluded that nonoxidative FFA disposal was higher in women. This increased flux could be an advantage at times of increased fuel needs but could also lead to metabolic abnormalities such as increased intracellular storage of TG in liver and muscle, which are associated with insulin resistance (27, 28). Plasma FFA are a major regulator of VLDL production with increased levels resulting in an increase in VLDL-TG production (29).
There was no correlation between nonoxidative FFA disposal and VLDL-TG PR. However, because a previous study has shown that the splanchnic uptake of FFA in healthy human subjects is only 27% of total FFA flux (30), this lack of correlation is not surprising. Using this figure for liver uptake of FFA, assuming that the percentage of palmitate oxidized in the liver is the same as the whole body and converting nonoxidative palmitate flux to nonoxidative FFA flux, if all VLDL-TG production was synthesized from imported FFA, this would account for 40% of nonoxidative FFA flux to the liver in men and 27% in women. However, this would be an overestimate because it has been shown that only 77% of VLDL-TG is derived from circulating fatty acids in the fasting state in healthy subjects (31). The fate of the remaining nonoxidative flux will include phospholipid synthesis and the esterification of cholesterol. It is unknown whether the flux rates for these differ in men and women.

Previous studies have shown VLDL-TG production by the liver to be higher in young obese men than young obese women, and because VLDL-TG clearance was similar in the two groups, this resulted in lower plasma TG in the women (6). In our study, there was also no difference between genders in VLDL1- and VLDL2-TG FCR. Total VLDL-TG PR was not different between genders. A previous study also reported that VLDL-TG PR was not different in postmenopausal obese women and men, although the men had plasma TG that were almost 0.5 mmol/liter higher than the women (25).

It is clear from this study that in postmenopausal women with the same plasma TG level as men, there is a difference in VLDL2-TG kinetics. This was due to both a higher hepatic VLDL2-TG PR and a higher transfer of VLDL1-TG to VLDL2-TG. Women have been shown to have higher lipoprotein lipase activity than men (32), and this may account for the higher transfer rate. Insulin has been shown to regulate the production of VLDL1 but not VLDL2 (7), suggesting there is independent regulation of these two VLDL particles. Although VLDL1 and VLDL2 apolipoprotein B concentration was not measured in our study, the higher TG pool was matched by a higher cholesterol pool in women; i.e. the TG to cholesterol ratio was similar in men and women, suggesting that there was an increase in the number of VLDL2 particles. The mechanism for the higher hepatic VLDL2 PR in women, which was a relatively small proportion of total VLDL-TG production, is not clear but may be related to the higher nonoxidative FFA flux. Although it is widely recognized that the availability of TG is an important regulator of VLDL production (29), both in vivo and in vitro studies have shown that cholesterol and/or cholesterol esters are also an important regulator of VLDL production (33).

Whether the higher VLDL2-TG PR relates to the mechanism for the higher risk of CVD in women with the same TG level as men is not clear, but the higher VLDL2-TG PR may generate more intermediate-density lipoprotein (IDL) and LDL. IDL and LDL levels have been shown to be associated with the progression of atherosclerosis in humans (34). Radioactively labeled VLDL2 has also been shown to be efficiently converted to LDL with more than 50% of tracer found in LDL after 24 h, whereas only 10% of VLDL1 was converted to LDL in the same time period (34). However, despite the higher VLDL2-TG PR in our study, LDL cholesterol was not different between genders. To gain a greater understanding of why high plasma TG are independent and better predictors of CVD in women, additional studies are needed to measure IDL and LDL kinetics in women and men matched for plasma TG.

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