VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity

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ALTERATIONS IN VERY LOW DENSITY lipoprotein (VLDL)-triglyceride (TG) metabolism are involved in the pathogenesis of coronary heart disease (CHD). Epidemiological studies have demonstrated a direct relationship between plasma TG concentration and the risk of CHD in both men and women (7, 10). In addition, an increase in plasma VLDL-TG concentration is associated with a decrease in plasma high density lipoprotein-cholesterol (10, 24) and an increase of low density lipoprotein particles (2, 10), which are also important risk factors for CHD (2, 6, 10).

Hepatic fatty acid availability is a major regulator of VLDL-TG production. In lean subjects, the majority (>75%) of fatty acids in VLDL-TG are derived from plasma (3, 34, 37), and increasing the delivery of plasma free fatty acids (FFA) to the liver stimulates the secretion of VLDL-TG in plasma (25). Glucose and insulin are also involved in the regulation of VLDL-TG secretion in plasma because of their effects on fatty acid metabolism. In lean subjects, both insulin (26–28) and glucose (1) acutely decrease VLDL-TG secretion predominantly by their suppressive effects on whole body lipolysis and plasma FFA availability. However, when the insulin-induced decrease in plasma FFA concentration is prevented by infusing Intralipid and heparin, insulin (i.e., hyperinsulimemic euglycemic clamp) suppresses VLDL-TG production (27), whereas glucose (with the concomitant increase in insulin concentration) stimulates VLDL-TG production (37).

We have recently found that both sex and obesity affect basal VLDL-TG metabolism in human subjects (32). The basal rate of VLDL-TG production was greater in lean women than in lean men but greater in obese men than obese women, because obesity was associated with increased VLDL-TG production in men but not in women despite elevated plasma VLDL-TG concentrations in both obese men and obese women (32). Little is known about the influence of sex or obesity on the insulin- and glucose-mediated effects on VLDL-TG metabolism. The effect of hyperinsulinemia on plasma VLDL-TG production has previously been evaluated in lean and obese women by using the hyperinsulinemic euglycemic clamp technique in conjunction with isotope tracer methods (26). The results of this study suggest that the relative decrease in VLDL-TG production rate during hyperinsulinemia is similar in lean and obese women. However, this conclusion may not be robust, because the VLDL-TG production rate was determined by using a tracer method that did not account for hepatic tracer recycling, which can have considerable effects on the calculation of VLDL-TG kinetics (35). We are not aware of any studies that evaluated the effect of increases in plasma insulin or glucose concentration on VLDL-TG metabolism in obese men.

The purpose of the present study was to evaluate the effect of glucose infusion on VLDL-TG kinetics in lean and abdominally obese men and women by using stable isotope tracer techniques in conjunction with mathe-
metrical modeling that can account for tracer recycling (35). We hypothesized that the glucose- and insulin-induced suppression of VLDL-TG production is blunted in obese compared with lean subjects because of insulin resistance to fatty acid metabolism.

**RESEARCH DESIGN AND METHODS**

**Subjects**

A total of 12 premenopausal women (6 lean, age 39 ± 4 yr, and 6 with abdominal obesity, age 39 ± 3 yr, waist circumference 100 ± 3 cm) and 12 men (6 lean, age 42 ± 2 yr, and 6 with abdominal obesity, age 41 ± 3 yr, waist circumference 107 ± 3 cm) participated in this study (Table 1). All subjects were considered to be in good health, except for obesity, after completing a history and physical examination, an electrocardiogram, and standard blood and urine tests. All subjects had normal oral glucose tolerance, and their plasma TG concentrations were within the normal range (0.6 ± 0.1 and 0.6 ± 0.1 mM in lean men and women, and 1.5 ± 0.2 and 1.7 ± 0.2 mM in obese men and women, respectively). None of the subjects was taking regular medications or smoked tobacco.

All subjects were weight stable for at least 2 mo before the study and had been sedentary (regular exercise <1 h/wk) for at least 6 mo before the study. Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine. Subjects were admitted to the inpatient unit of the GCRC at Washington University School of Medicine the day before the isotope infusion study. At 1900, subjects consumed a standard meal containing 12 kcal/kg adjusted body wt for obese subjects. Adjusted body weight was calculated as ideal body weight (midpoint of the medium frame of the 1983 Metropolitan Life Insurance Table) + 0.25 × (actual body weight – ideal body weight) to eliminate marked differences in energy balance (energy intake – energy expenditure) between lean and obese individuals. The meal consisted of 55% of total energy carbohydrates, 30% fat, and 15% protein. At 2000, the subjects ingested a liquid formula (Ensure; Ross Laboratories, Columbus, OH), containing 250 kcal (40 g carbohydrates, 6.1 g fat, and 8.8 g protein), and then fasted until completion of the study the next day (basal study). For the glucose study, glucose infusion (5.5 mg·kg FFM⁻¹·min⁻¹) in an antecubital vein was started at 2100 and maintained until the completion of the isotope infusion study the next day (1900). The infusion rate of glucose was adjusted to each subject’s FFM because lean body mass (i.e., skeletal muscle) is the major site of basal glucose utilization (15) and insulin-mediated glucose uptake (21).

The next morning, at 0530, one catheter was inserted in a forea vein to administer stable isotope-labeled glycerol, and a second catheter was inserted in a contralateral hand vein, which was heated to 55°C by using a thermostatically controlled box, to obtain arterIALIZED blood samples. At 0700 (time 0), after a blood sample for determination of plasma substrate (glucose, fatty acid, and TG) and insulin concentrations and the background glycerol tracer-to-tracee ratio (TTR) in plasma and VLDL-TG was obtained, a bolus of [1,1,2,3,3-²H₅]glycerol (50 μmol/kg; Cambridge Isotope Laboratories, Andover, MA), dissolved in 0.9% saline, was administered through the catheter in the forearm vein. Blood samples were obtained at 5, 15, 30, 45, and 60 min and then every hour for 12 h after the tracer injection to determine the glycerol TTR in plasma and VLDL-TG and plasma substrate concentrations. Blood samples for the determination of plasma insulin concentrations were collected at 2 and 12 h. Subjects remained in bed for the entire duration of the study.

**Sample Collection**

Blood samples for the determination of plasma fatty acid concentrations and glycerol TTR in plasma and VLDL-TG were collected in chilled tubes containing EDTA. Blood samples for the determination of insulin concentration were collected in chilled tubes containing EDTA and trasylol. The samples were placed in ice, and plasma was separated by centrifugation within 30 min of collection. Aliquots of plasma (−2 ml) were kept in the refrigerator for subsequent isolation of VLDL. The remaining plasma samples were stored at −70°C until final analyses were performed. Blood samples for the determination of plasma glucose concentration were collected in tubes containing heparin, centrifuged for 30 s, and analyzed immediately after collection.

**Isolation of Lipoproteins**

Immediately after the completion of the study, VLDL was isolated by ultracentrifugation (9). Briefly, 1.5 ml of each plasma sample was transferred to Optiseal tubes (Beckman Instruments, Palo Alto, CA), covered with a saline solution (density = 1.006 kg/l), and centrifuged (50.4 Ti rotor; Beckman Instruments) for 16 h at 100,000 g and 10°C. The top layer, containing VLDL, was removed by tube slicing (Beckman Instruments). The exact volume that was recovered (−3 ml) was recorded, and samples were stored at −70°C until final analyses were performed.

**Sample Analyses**

Plasma glucose concentration was determined on an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs Instruments, Ohio).
Springs, OH). Plasma insulin concentration was measured by RIA (13). For each subject, plasma insulin concentrations during basal conditions and during glucose infusion were measured simultaneously by using the same insulin assay and run. The intra-assay variability for the determination of insulin concentration was <10%. Total plasma TG and VLDL-TG concentrations were measured using a spectrophotometric enzymatic kit (Sigma Chemicals, St. Louis, MO). Plasma fatty acid concentrations were quantified by gas chromatography (model 5890-II; Hewlett-Packard, Palo Alto, CA) after heptadecanoic acid was added to plasma as an internal standard (30).

Plasma free glycerol TTR was determined by GC-MS (MSD 5973 system; Hewlett-Packard), as previously described (16). Plasma proteins were precipitated with ice-cold acetone, and hexane was used to extract plasma lipids. The aqueous phase was dried by centrifugation under vacuum (SpeedVac; Savant Instruments, Farmingdale, NY), and heptafluorobutyric (HFB) anhydride was used to form an HFB derivative of glycerol. Ions were produced by electron impact ionization, and glycerol TTR was determined by mass spectrometry monitoring ions at mass-to-charge ratios (m/z) of 467 and 472.

Calculations

The fractional catabolic rate (FCR) of VLDL-TG, which represents the fraction of the VLDL-TG pool that disappears from plasma per unit of time, was determined by fitting the glycerol TTR time course in plasma and in VLDL-TG to a multicompartmental model, as previously described (35). The values for FCR are expressed in pools per hour. It was assumed that the rate of VLDL-TG production was equal to the rate of VLDL-TG catabolism, because plasma VLDL-TG concentration remained constant throughout the 12-h sampling period. The absolute rate of VLDL-TG production (equal to the absolute rate of VLDL-TG catabolism) was calculated as 1) total VLDL-TG secretion rate, which represents the amount of VLDL-TG produced by the liver and secreted in the bloodstream, and 2) VLDL-TG secretion rate per unit of plasma, which represents the rate of secretion of VLDL-TG in the bloodstream as follows:

\[
\text{total VLDL-TG secretion rate (in } \mu\text{mol/min}) = \frac{(\text{VLDL-TG FCR} \times C_{\text{VLDL-TG}} \times PV)}{60}
\]

\[
\text{VLDL-TG secretion/l plasma (in } \mu\text{mol/l plasma }^{-1}\text{-min}^{-1}) = \frac{(\text{VLDL-TG FCR} \times C_{\text{VLDL-TG}})}{60}
\]

where \(C_{\text{VLDL-TG}}\) is the concentration of VLDL-TG in plasma, and \(PV\) is the plasma volume, which was estimated based on each subject’s FFM (PV = 0.551 × kg FFM; see Refs. 5 and 11). It was assumed that PV was equal to the VLDL-TG volume of distribution, because VLDL is restricted to the plasma compartment and does not enter the interstitial space or the lymphatic system (36). Total VLDL-TG secretion rate was also expressed normalized to body weight (in \(\mu\text{mol·kg body wt}^{-1}\text{-min}^{-1}\)) to account for the potential confounding of differences in total body mass between lean and obese subjects, and men and women.

VLDL-TG clearance from plasma (ml/min) was calculated by dividing the rate of VLDL-TG disappearance from plasma (VLDL-TG catabolic rate in \(\mu\text{mol/min}\)) by the plasma VLDL-TG concentration (in \(\mu\text{mol/ml}\)).

Statistical Analysis

A two-way ANOVA with repeated measures (adiposity × treatment) was performed to test the significance of differences in VLDL-TG kinetics in the basal state and during glucose infusion within each sex. Significant differences between groups (F-ratios) were followed by Tukey’s analysis. Plasma VLDL-TG concentration and total VLDL-TG production data were not normally distributed, so the data were logarithmically transformed to analyze the significance of differences between groups. All analyses were carried out with SigmaStat version 2.3 (SPSS, Chicago, IL). A value of \(P < 0.05\) was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Glucose Infusion Rate and Plasma Glucose and Insulin Concentrations

Basal plasma glucose concentration was similar in men and women, and in lean and obese subjects. The total amount of glucose that was infused during the study was 454 ± 11 g in lean and 492 ± 40 g in obese men, and 302 ± 10 g in lean and 331 ± 7 g in obese women. Glucose infusion raised \((P < 0.001)\) plasma glucose concentration by ~40% compared with basal conditions in lean men, lean women, and obese women and by ~55% in obese men (Fig. 1).

Basal plasma insulin concentrations were significantly higher in obese than in lean women \((P < 0.001)\) but not in obese compared with lean men; failure to observe a difference between lean and obese men may reflect a type II statistical error because of the small number of study subjects. Glucose infusion raised plasma insulin concentration two- to threefold \((P < 0.01)\) compared with basal values in all subjects (Fig. 2). Under basal conditions, plasma insulin concentrations decreased by ~20% from the beginning to the end of the study (0–12 h). The decrease tended to be greater in lean than in obese subjects; however, this difference was not statistically significant. Plasma insulin concentrations were constant during the entire study period during glucose infusion.

Plasma Fatty Acid and VLDL-TG Concentrations

During basal conditions, plasma fatty acid concentration gradually increased during the 12-h study period. Glucose infusion decreased plasma fatty acid concentration to approximately one-half of basal values, and plasma fatty acid concentration remained constant throughout the study (Fig. 1). Plasma VLDL-TG concentration was higher in obese than in lean subjects, both during basal conditions and during glucose infusion. During glucose infusion, VLDL-TG concentration...
was marginally lower compared with basal values, but the difference did not reach statistical significance (Fig. 3). Both during basal conditions and during glucose infusion, plasma VLDL-TG concentrations were constant throughout the entire study period (data not shown).

**VLDL-TG Kinetics**

The compartmental model fit our experimental data in all subjects during basal conditions and during glucose infusion. The time course of VLDL-TG enrichment that results from a bolus injection of [2H5]glycerol in lean and obese men and women has been presented previously (32, 35).

**Lean and obese men.** Basal VLDL-TG FCR was not different between lean and obese men; glucose infusion decreased VLDL-TG FCR ($P < 0.01$), and there was no difference in the response between lean and obese men (Table 2). The basal rate of VLDL-TG secretion in plasma was more than double in obese than in lean men. Glucose infusion decreased ($P < 0.001$) the rate of total VLDL-TG secretion to approximately one-half of basal values in lean and obese men; thus, VLDL-TG secretion remained higher ($P < 0.003$) in obese than lean men during glucose infusion (Figs. 4 and 5). The clearance rate of VLDL-TG from plasma during basal conditions was not different in lean and obese men (Table 2). During glucose infusion, plasma clearance of VLDL-TG was $\sim 30\%$ lower ($P < 0.01$) compared with basal values in both lean and obese men (Table 2).

**Lean and obese women.** Basal VLDL-TG FCR in lean women was more than double the value observed in obese women ($P < 0.01$). Glucose infusion decreased VLDL-TG FCR in all lean women ($P < 0.01$) but only in three of the six obese women; it increased in the other three (Table 2). The basal rate of VLDL-TG secretion in plasma was not different in obese compared with lean women. Glucose infusion decreased the rate of total VLDL-TG secretion by $\sim 55\%$ in lean women ($P < 0.02$); it was not different from basal values in obese women (Figs. 4 and 5). The clearance rate of VLDL-TG from plasma during basal conditions was higher in lean than in obese women ($P < 0.05$). Glucose infusion reduced plasma clearance of VLDL-TG by $\sim 50\%$ in lean women ($P < 0.01$) but had no effect on VLDL-TG clearance in obese women (Table 2).

**DISCUSSION**

Decreasing endogenous VLDL-TG production during postprandial conditions is important for regulating whole body TG flux when dietary TG are entering the systemic circulation via chylomicrons. The suppression of VLDL-TG production during feeding is mediated, in large part, by glucose-induced stimulation of insulin secretion, which inhibits adipose tissue lipolysis (12) thereby decreasing fatty acid delivery to the liver. In this study, we examined the effect of hyperglycemia-hyperinsulinemia, induced by glucose infusion, on VLDL-TG metabolism in lean and obese men and women. Glucose was infused at a rate designed to achieve similar plasma glucose concentrations in all groups, similar to that in the postprandial state. Glucose infusion caused a marked decrease in VLDL-TG production in lean and obese men and lean women, but not in obese women. The blunted response in obese women occurred despite similar plasma fatty acid concentrations to those in the other groups. These data demonstrate that sex and adiposity affect the glucose-
and/or insulin-mediated suppression of VLDL-TG production and suggest that plasma fatty acid availability may, at least in obese women, not be the primary regulator of VLDL-TG metabolism.

The suppression of VLDL-TG production during glucose infusion was probably related to the glucose-induced hyperinsulinemia, because insulin is a potent inhibitor of lipolysis of adipose tissue TG (12, 14, 17). Data from several studies suggest that VLDL-TG production is largely controlled by hepatic fatty acid availability (1, 26–28, 37). The availability of fatty acids for VLDL-TG production depends on fatty acid delivery to the liver from peripheral and intrahepatic and intra-peritoneal adipose tissue TG. Insulin, however, may also inhibit VLDL-TG production by a mechanism that is independent of its effect on fatty acid availability. It was found that insulin infusion suppressed VLDL-TG production even when the insulin-mediated decrease in fatty acid availability is prevented by infusing Intralipid and heparin (27). The direct suppressive effect of insulin on VLDL-TG production has also been demonstrated in studies in rats (8).

The mechanism(s) responsible for the impaired suppression of VLDL-TG production during glucose infusion observed in our obese women is not clear. During basal conditions, in lean subjects, the majority (>75%) of fatty acids in VLDL-TG are derived from the systemic circulation (3, 34, 37), whereas in obese subjects, approximately one-half of the fatty acids in VLDL-TG are derived from the systemic circulation (3); the remaining fatty acids are presumably derived from lipolysis of intraperitoneal and intrahepatic TG, because de novo synthesized fatty acids contribute <5% to total hepatic VLDL-TG production in the basal postabsorptive state (1, 29). The relative contribution of de novo synthesized fatty acids to total VLDL-TG production during hyperglycemia-hyperinsulinemia increases during hyperglycemia-hyperinsulinemia (1) and conditions that favor hepatic lipogenesis (20). The extent to which this occurs is quite variable; however, it does not appear to be affected by adiposity (20, 29). Glucose infusion caused a marked but similar decrease in plasma fatty acid concentrations in lean and obese women, so systemic fatty acid delivery to the liver was
probably the same in both groups. It is possible that fatty acids derived from lipolysis of intraperitoneal and intrahepatic TG maintained VLDL-TG production rates during glucose infusion in our obese women. However, the relative suppression of VLDL-TG production during glucose infusion in our obese and lean men was similar, even though it is likely that our obese men had greater amounts of intraperitoneal and intrahepatic TG than our lean men (23). Furthermore, although the relative increase in plasma insulin concentration during glucose infusion tended to be higher in our men than in our women, the insulin response was similar in lean and obese subjects of the same sex. Therefore, it is unlikely that insulin per se was responsible for the differences in VLDL-TG production observed between our obese men and obese women in response to glucose infusion, unless the sensitivity of VLDL-TG metabolism to insulin is diminished in obese women. However, men and obese subjects are usually less sensitive than women and lean subjects to the effects of insulin on glucose and fatty acid metabolism (33, 38). This suggests that other factors are involved in regulating VLDL-TG production, which may be responsible for the blunted suppression of VLDL-TG production in response to hyperglycemia-hyperinsulinemia in obese women.

We are aware of only one study that has investigated the effect of insulin on VLDL-TG metabolism in obese

Table 2. VLDL-TG kinetics

<table>
<thead>
<tr>
<th></th>
<th>Lean Basal</th>
<th>Lean Glucose</th>
<th>Obese Basal</th>
<th>Obese Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR, pools/h</td>
<td>0.53 ± 0.08</td>
<td>0.30 ± 0.06*</td>
<td>0.44 ± 0.05</td>
<td>0.32 ± 0.04*</td>
</tr>
<tr>
<td>Total VLDL-TG secretion, µmol/min</td>
<td>10 ± 1</td>
<td>5 ± 1*</td>
<td>30 ± 3†</td>
<td>16 ± 2†</td>
</tr>
<tr>
<td>VLDL-TG secretion, µmol·kg body wt⁻¹·min⁻¹</td>
<td>0.13 ± 0.02</td>
<td>0.07 ± 0.02*</td>
<td>0.30 ± 0.03†</td>
<td>0.16 ± 0.03†</td>
</tr>
<tr>
<td>VLDL-TG clearance, ml plasma/min</td>
<td>31 ± 5</td>
<td>18 ± 4*</td>
<td>29 ± 4</td>
<td>21 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. FCR, fractional catabolic rate; VLDL, very low density lipoprotein; TG, triglyceride. *Value significantly different from corresponding value during basal conditions (P < 0.05). †Value significantly different from corresponding value in lean subjects (P < 0.05). a Value represents the total amount of VLDL-TG secreted by the liver and does not take into account differences in total body mass between lean and obese subjects and between men and women. b Value represents the total rate of VLDL-TG secreted by the liver normalized to body weight.

Fig. 4. VLDL-TG secretion rate in the basal, postabsorptive state and during glucose infusion (5.5 mg·kg FFM⁻¹·min⁻¹) in lean and obese men and lean and obese women. P < 0.05: *value significantly different from corresponding value during basal conditions; †value significantly different from corresponding value in lean subjects. All values are means ± SE.

Fig. 5. Glucose-induced change in VLDL-TG secretion rate in lean and obese men and lean and obese women. *Value significantly different (P < 0.01) from zero. All values are means ± SE.
individuals, and that study was conducted only in women (26). In contrast to our findings, Lewis et al. (26) found that the insulin-induced suppression of VLDL-TG production was similar in lean and obese women. However, the method used to assess VLDL-TG kinetics in that study did not account for hepatic tracer recycling, which can have a considerable effect on the calculation of VLDL-TG kinetics (35). Studies that investigated the effect of insulin on plasma VLDL-TG concentration support our findings that the insulin-induced suppression of VLDL-TG production in obese women is lower than in obese men and lean subjects. Lewis et al. (26) found that plasma VLDL-TG concentration decreased in response to hyperinsulinemia in lean but not in obese women (26). Bioletto et al. (4), who compared a group of obese men and women with lean men only found that the insulin-induced decrease in plasma VLDL-TG concentration was blunted in the obese compared with the lean subjects. In our study, plasma VLDL-TG concentration was only marginally affected by glucose infusion, probably because the plasma insulin concentrations during glucose infusion were ~50% lower compared with those achieved during insulin clamps in the other studies. In addition, our findings are in accordance with studies that investigated the effect of obesity, but not sex, on VLDL-TG kinetics during consumption of fat-free liquid formulas and found that VLDL-TG production was higher in obese than lean subjects (11, 18, 19, 22). It is possible that we missed a small effect of glucose on VLDL-TG secretion in our obese women due to a type II statistical error. However, even if this is the case, our conclusion that the effect of glucose infusion on VLDL-TG secretion is blunted in obese women compared with lean women, lean men, or obese men is likely correct.

In summary, the data from the present study demonstrate that sex and obesity have independent effects on the regulation of VLDL-TG production. Glucose infusion decreased the rate of VLDL-TG production in lean individuals and obese men, but not in obese women, despite similar plasma fatty acid concentrations and, presumably, systemic plasma fatty acid availability in all subjects. The mechanism(s) that is responsible for these differences is not known. These results underscore the importance of controlling for sex in future studies evaluating lipid metabolism in human subjects.

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