Increased VLDL-Triglyceride Secretion Precedes Impaired Control of Endogenous Glucose Production in Obese, Normoglycemic Men

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OBJECTIVE—To assess basal and insulin-mediated VLDL-triglyceride (TG) kinetics and the relationship between VLDL-triglyceride secretion and hepatic insulin resistance assessed by endogenous glucose production (EGP) in obese and lean men.

RESEARCH DESIGN AND METHODS—A total of 12 normoglycemic, obese (waist-to-hip ratio >0.9, BMI >30 kg/m^2) and 12 lean (BMI 20–25 kg/m^2) age–matched men were included. Ex vivo–labeled [1-^{14}C]VLDL-TGs and [3-^{3}H]glucose were infused postabsorptively and during a hyperinsulinemic-euglycemic clamp to determine VLDL-TG kinetics and EGP. Body composition was determined by dual X-ray absorptiometry and computed tomography scanning. Energy expenditure and substrate oxidation rates were measured by indirect calorimetry.

RESULTS—Basal VLDL-TG secretion rates were increased in obese compared with lean men (1.25 ± 0.34 vs. 0.86 ± 0.34 μmol/kg fat-free mass [FFM]/min; P = 0.011), whereas there was no difference in clearance rates (150 ± 56 vs. 162 ± 77 ml/min; P = NS), resulting in greater VLDL-TG concentrations (0.74 ± 0.40 vs. 0.38 ± 0.20 mmol/L; P = 0.011). The absolute insulin-mediated suppression of VLDL-TG secretion was similar in the groups. However, the percentage reduction (−36 ± 18 vs. −54 ± 10%; P = 0.008) and achieved VLDL-TG secretion rates (0.76 ± 0.20 vs. 0.41 ± 0.19 μmol/kg FFM/min; P < 0.001) were impaired in obese men. Furthermore, clearance rates decreased significantly in obese men, but there was no significant change in lean men (−17 ± 18 vs. 7 ± 20%; P = 0.007), resulting in less percentage reduction of VLDL-TG concentrations in obese men (−22 ± 20 vs. −56 ± 11%; P < 0.001). Insulin-suppressed EGP was similar (0.4 mg/kg FFM/min [0.0–0.8] vs. 0.1 mg/kg FFM/min [0.0–1.2]; P = NS), and the percentage reduction was equivalent (−80% [57–98] vs. −98% [49–100], P = NS). Insulin-mediated glucose disposal was significantly reduced in obese men.

CONCLUSIONS—Basal VLDL-TG secretion rates are increased in normoglycemic but insulin-resistant, obese men, resulting in hypertriglyceridemia. Insulin-mediated suppression of EGP is preserved in obese men, whereas suppression of VLDL-TG secretion is less pronounced in obese men. Compared with EGP, the inability to achieve suppression of VLDL-triglyceride secretions to a level similar to control subjects during hyperinsulinemia seems to be an early manifestation in male obesity.

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Insulin-resistant conditions, including obesity and type 2 diabetes, are associated with plasma lipid abnormalities comprising hypertriglyceridemia, low HDL cholesterol concentrations, small and dense LDL particles, and excessive postprandial lipemia (1–4). These atherogenic lipid abnormalities often precede both impaired glycemic control and overt type 2 diabetes by several years, indicating that altered lipoprotein metabolism is an early event in the development of insulin resistance. Evidence suggests that increased secretion of VLDL–triglycerides (TG), primarily in the subfraction of large, TG-rich VLDL particles ($VLDL_1$), is an early feature of insulin-resistant dyslipidemia, as reviewed in detail elsewhere (5,6).

The impact of male obesity on VLDL-TG and VLDL–apolipoprotein B (apoB) kinetics has been examined in relatively few studies (7–10). In studies by Mittendorfer et al. (7,8), basal VLDL-TG secretion rates into plasma were increased by >150% in obese compared with lean men, with no significant difference in clearance rates, resulting in ~150% greater VLDL-TG concentrations. Correspondingly, in studies by Chan et al. (9,10), VLDL-apoB secretion rates were increased in obese men. To our knowledge, the suppressive effect of acute hyperinsulinemia on hepatic VLDL-TG secretion has not been studied previously in obese men. In nonobese men, acute hyperinsulinemia significantly decreased hepatic secretion of VLDL-TG (11) and VLDL-apoB (12,13). Moreover, Mittendorfer et al. (8) explored physiological insulin surges during glucose infusion and found that increased glucose levels acutely decrease VLDL-TGsecretion into plasma by ~50% and VLDL-TGclearance by ~30% in both obese and lean men. However, with this design, it is not possible to distinguish between the separate effects of hyperglycemia and concomitant hyperinsulinemia on VLDL-TG kinetics.

In healthy individuals, endogenous glucose production (EGP) is suppressed by insulin after a meal, whereby normoglycemia is maintained. This ability to maintain postabsorptive glucose homeostasis is vital, and insulin-mediated reduction in EGP has, therefore, traditionally been used as a surrogate measure of hepatic insulin sensitivity. Likewise, insulin-mediated suppression of VLDL-TG secretion is appropriate after food ingestion to facilitate the rapid clearance of excess TG from circulation. However, although EGP is effectively regulated by insulin in the portal vein (secreted in proportion to plasma glucose concentration), it is more unclear what regulates postabsorptive lipid homoeostasis. Mechanistic studies have demonstrated that both indirect free fatty acid (FFA)-dependent and direct FFA-independent mechanisms (11,12) are involved in

the short-term regulation of VLDL-TG secretion. None have established whether obese individuals lose the suppressive effect of insulin on VLDL-TG secretion before its ability to suppress EGP.

The purpose of this study was to compare normoglycemic, obese and lean men and to assess as follows *1*) postabsorptive VLDL-TG kinetics and *2*) the effect of hyperinsulinemia on VLDL-TG metabolism and EGP. We hypothesized that impaired suppression of hepatic VLDL-TG secretion precedes impaired glycemic control in obese men.

RESEARCH DESIGN AND METHODS

The study was approved by the local ethics committee, and informed consent was obtained from all participants. A total of 12 obese (waist-to-hip ratio >0.9, BMI >30 kg/m^2) and 12 lean (BMI 20–25 kg/m^2) age–matched men were recruited through local advertisements. All were nonsmokers and in good health, and none used any medications. In both groups, some of the subjects were recreationally active, but none were elite-trained. By protocol, all had normal fasting plasma glucose, blood count, and liver and kidney function.

Potentially eligible subjects visited the clinical research laboratories after an overnight 10–12-h fast for a screening visit that included blood sampling. One week before the study day, subjects who met the eligibility criteria visited again after an overnight fast. Blood was drawn for the VLDL-TG ex vivo–labeling procedure, as described below. Dual X-ray absorptiometry scans and abdominal computed tomography scans at the L_2–L_3 interspace were performed to obtain measures of body composition. Finally, volunteers were interviewed by a dietitian to estimate daily caloric intake. A weight-maintaining diet (55% carbohydrate, 15% protein, and 30% fat) was provided by the hospital kitchen during the 3 days preceding the metabolic study.

Volunteers were admitted at 2200 h the evening before the study for an overnight stay. From this time until the end of the study, they remained in bed, with the exception of voiding, and fasted, with the exception of tap water. The study protocol is illustrated in Fig. 1. At 0700 h (t = −30 min), an intravenous catheter was placed in an antecubital vein for infusions and another in a dorsal hand vein for blood sampling. The hand was placed in a heated box to obtain arterialized blood. At 0730 h (t = 0), primed-continuous infusions of ex vivo–labeled [1-^{14}C]VLDL-TG tracer (20% priming dose, the remaining volume as a continuous infusion) and [3-^{3}H]glucose (Lægemiddelstyrelsen Isotop Apoteket, Denmark) (12 μCi as a priming dose, 12 μCi/min as a continuous infusion) was started. From t = 150 min, human insulin (Actrapid; Novo Nordisk) was infused at a rate of 0.5 μU/kg fat-free mass (FFM)/min. Plasma glucose was measured every 10 min and clamped at ~5 mmol/L by variable infusion of 20% glucose. During the glucose clamp, [3-^{3}H]glucose was added to the glucose infusate to avoid rapid dilution of plasma [^{3}H]glucose specific activity (SA) (14). Blood samples to determine insulin and metabolite concentrations were drawn at t = 0, 60, 120, and 150 min (basal period) and at 210, 270, 330, 390, and 420 min (clamp period), whereas samples to determine [^{14}C]VLDL-TG and [^{3}H]glucose SA were drawn at baseline and at 10-min intervals during the last 30 min of each period (basal and clamp steady-state periods). Indirect calorimetry was performed in the same time intervals. ApoB concentration was determined in samples drawn at 150 and 420 min. At t = 420 min, all infusions were stopped and catheters removed, with the exception of for isotonic glucose. Volunteers were served lunch, and after ensuring stable plasma glucose for at least 30 min, they were discharged.

VLDL-TG tracer preparation. The ex vivo–labeling procedure of VLDL-TG with radiolabeled triolein has previously been described in detail (15). In brief, plasma from a 60-mL blood sample was mixed with ~30 μCi [1-^{14}C]triolein (PerkinElmer Life and Analytical Sciences) dissolved in 300 μL ethanol and sonicated in a cell incubator at 37°C for 6 h. The labeled plasma was then transferred to sterile OptiSeal centrifuge tubes (Beckman Instruments), covered with a saline solution (d = 1.006 g/mL), and centrifuged (Ti 50.3 rotor; Beckman Instruments) for 18 h at 40,000 rpm and 10°C. The supernatant containing the labeled VLDL particles was removed using a sterile Pasteur pipette, filtered, and stored at 5°C. Samples were tested for bacterial growth to ensure sterility.

Body composition and visceral fat. Body composition was determined by dual X-ray absorptiometry scan (QDR-2000; Hologic). The fat mass of the upper body, abdominal, and lower body was determined using the region-of-interest program. Computed tomography scans were used to determine the ratio of visceral to total abdominal fat. Visceral fat mass was estimated by multiplying this ratio by total abdominal fat mass (16). Upper-body subcutaneous fat mass was calculated by subtracting visceral fat mass from total upper-body fat mass.

Indirect calorimetry. Energy expenditure and substrate oxidation rates were measured by indirect calorimetry (Deltatrac monitor; Datex Instruments). Lipid and glucose oxidation rates were calculated using the nonprotein respiratory quotient (17).

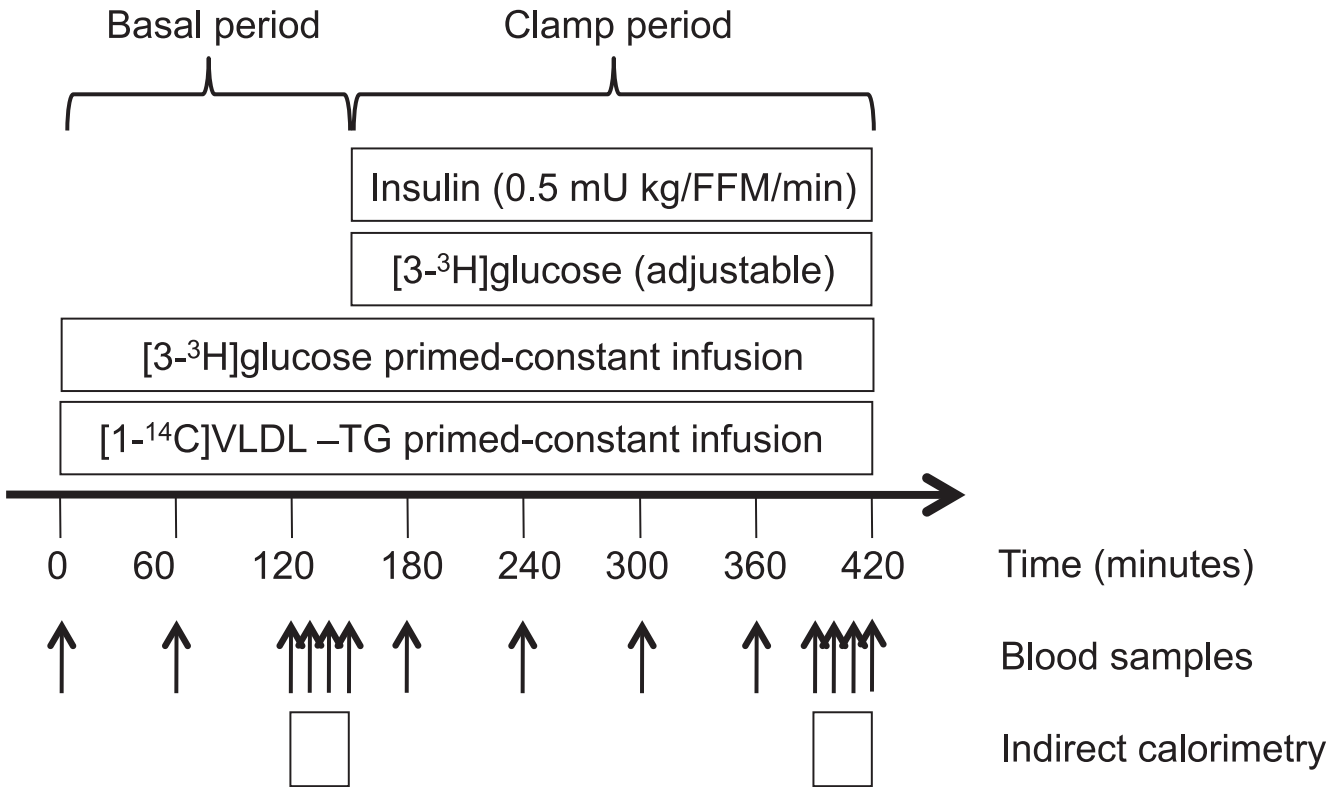

FIG. 1. Study protocol.

Laboratory procedures. Plasma glucose concentrations were measured immediately using a YSI 2300 STAT Plus glucose analyzer (YSI). Blood samples were placed on ice and separated as quickly as possible by centrifugation (3,600 rpm at 4°C for 10 min). Aliquots of plasma (~3 mL) were stored at 4°C for isolation of VLDL after completion of the examination, as described below. Remaining samples were stored at −20°C for later analysis. [^{3}H]Glucose SA was determined as described previously (14). TG concentrations were analyzed using a COBAS Fara II (F. Hoffmann–La Roche). Serum insulin concentrations were measured using an immunoassay (DAKO Denmark). Serum FFA concentrations were determined by a colorimetric method (Wako Pure Chemical Industries). VLDL-apoB concentrations were determined using an enzyme-linked immunosorbent assay kit (Mabtech). Duplicate samples were diluted to ensure reading on the linear part of the standard curve.

Plasma VLDL-TG SA. VLDL was isolated from ~3 mL of each plasma sample by ultracentrifugation, as described above. The supernatant containing the VLDL fraction (~1.2 mL) was obtained by tube slicing (Beckman Instruments) and transferred to a scintillation vial. A 300-μL sample was analyzed for TG concentration. VLDL-TG quantity (μmol) in the sample and plasma concentration (mmol/L) was calculated. Scintillation fluid (Optiphase HiSafe 2; PerkinElmer Life and Analytical Sciences) was added, and ^{14}C activity was counted to a <2% counting error. VLDL-TG SA was expressed as disintegrations per minute per micromoles.

Calculations

VLDL-TG kinetics. VLDL-TG SA steady state was effectively reached during the 30-min steady-state periods at the end of the basal and the clamp periods. VLDL-TG secretion rates (micromoles per minute) were calculated by dividing the infusion rate (F) by the plateau SA in each period:

$$\text{VLDL-TG secretion rate} = \frac{F}{SA}$$

VLDL-TG clearance rates (milliliters per minute) were calculated by dividing the secretion rate by the average VLDL-TG concentration ($C_{VLDL\text{-}TG}$) in each period:

$$\text{VLDL-TG clearance rate} = \frac{\text{VLDL-TG secretion rate}}{C_{VLDL\text{-}TG}}$$

Glucose kinetics. Glucose turnover rates (milligrams per minute) were calculated at 10-min intervals during the 30-min steady–state periods using Steele's non–steady-state equations (18). During the clamp period, EGP was calculated by subtracting the rate of exogenous glucose infusion from the glucose rate of appearance (R_a). Net lipid and glucose oxidation rates were computed from the indirect calorimetry measurements, and protein oxidation rates were estimated from urinary carbamide excretion. Net nonoxidative glucose disposal was calculated by subtracting the glucose oxidation rate from the isotopically determined glucose disposal rate (R_d).

Statistics. All kinetic and oxidation rates are corrected for FFM except VLDL-triglyceride clearance rates, which are expressed as milliliters per minute. Concentrations are expressed as the average concentration during the 30-min steady-state periods at the end of the basal and the clamp periods. Data are presented as means ± SD or median (range). Between-group comparisons at baseline and during clamp were performed using the Student t test or Mann-Whitney test for normal and nonnormal distributed data, respectively. Two-way ANOVA for repeated measurements were used to compare basal with clamp changes between lean and obese subjects. Within-group comparisons were performed using the Student t test for paired comparisons or the Wilcoxon test for normal and nonnormal distributed data, respectively. Correlations were tested using Pearson r or Spearman ρ. If both variables in the analysis were corrected for FFM, uncorrected values were used. A $P < 0.05$ was considered significant.

RESULTS

Subject characteristics. Subject characteristics are summarized in Table 1. BMI was 32.0 ± 1.1 kg/m^2 in obese men and 22.6 ± 1.7 kg/m^2 in lean men ($P < 0.001$), and the groups were well matched for age. As expected, the groups differed with respect to body composition. By protocol, all subjects were normoglycemic. Of note, the obese men had hypertriglyceridemia and decreased HDL cholesterol concentrations.

Insulin, glucose, and FFAs. Concentrations of insulin, glucose, and FFAs are shown in Fig. 2. Basal concentrations of insulin were increased in obese compared with lean men (46 ± 17 vs. 22 ± 8 pmol/L; $P < 0.001$), whereas there were no significant differences in glucose (5.1 ± 0.2 vs. 5.0 ± 0.3 mmol/L; $P = 0.063$) and FFA concentrations (0.49 ± 0.16 vs. 0.51 ± 0.10 mmol/L; $P = 0.402$). Insulin infusion led to a comparable increase in insulin concentration ($P = 0.221$), resulting in greater concentrations in obese men (196 ± 38 vs. 156 ± 35 mmol/L; $P = 0.014$). Euglycemia was maintained throughout the clamp (5.0 ± 0.1 vs. 5.0 ± 0.2 mmol/L; $P = 0.895$). The insulin-mediated suppression in FFA concentration was impaired in obese men (−78 ± 8% vs. −92 ± 4%; $P < 0.001$), resulting in greater FFA concentrations (0.11 ± 0.04 vs. 0.04 ± 0.02 mmol/L; $P < 0.001$).

TABLE 1
Subject characteristics

	Obese men	Lean men	P
Age (years)	27 (22–49)	28 (22–44)	0.931
Weight (kg)	108.8 ± 8.5	76.4 ± 4.2	<0.001
BMI (kg/m^2)	32.0 ± 1.1	22.6 ± 1.7	<0.001
Fat percentage (%)	26.5 ± 3.4	15.1 ± 3.8	<0.001
Fat mass (kg)	28.4 ± 4.8	11.3 ± 2.8	<0.001
Visceral fat (kg)	3.5 ± 0.9	1.1 ± 0.4	<0.001
Upper-body subcutaneous fat (kg)	13.4 ± 2.5	4.2 ± 1.2	<0.001
Lower-body fat (kg)	10.2 ± 2.2	4.8 ± 1.5	<0.001
FFM (kg)	78.7 ± 6.0	64.1 ± 5.0	<0.001
Glucose (mmol/L)	5.1 ± 0.2	5.0 ± 0.3	0.063
Insulin (pmol/L)	57.5 ± 12.9	25.3 ± 11.1	<0.001
FFAs (mmol/L)	0.481 ± 0.199	0.509 ± 0.150	0.704
TG (mmol/L)	1.3 (0.6–2.3)	0.8 (0.5–1.4)	0.009
Total cholesterol (mmol/L)	4.8 ± 0.8	4.7 ± 0.8	0.897
LDL cholesterol (mmol/L)	2.8 ± 0.7	2.9 ± 0.7	0.815
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.3 ± 0.3	0.031

Data are means ± SD or median (range).

VLDL-TG kinetics. Plasma [^{14}C]VLDL-TG SA steady state was reached in the basal and clamp steady-state periods (Fig. 3*B*). VLDL-TG secretion rates were increased in obese men in both the basal state (1.25 ± 0.34 vs. 0.86 ± 0.34 μmol/kg FFM/min; $P = 0.011$) and during hyperinsulinemia (0.76 ± 0.20 vs. 0.41 ± 0.19 μmol/kg FFM/min; $P < 0.001$). The suppression in secretion during hyperinsulinemia was significant ($P < 0.001$) in both groups. The absolute change was similar (0.49 ± 0.30 vs. 0.46 ± 0.19 μmol/kg FFM/min; P = NS). However, the relative suppression was impaired in obese men compared with lean men (−36 ± 18 vs. −54± 10%; $P = 0.008$) (Fig. 3*C* and *E*). VLDL-TG clearance rates were comparable both in the basal period (150 ± 56 vs. 162 ± 77 ml/min; $P = 0.931$) and during the clamp (171 ± 79 vs. 124 ± 52 ml/min; $P = 0.097$). Although clearance decreased in obese men ($P = 0.011$), there was no change in lean men ($P = 0.329$). The absolute ($P = 0.009$) and the relative change was different in the two groups (−17 ± 18 vs. 7 ± 20%; $P = 0.007$) (Fig. 3*D* and *F*).

VLDL-TG concentrations were increased in obese men in both the basal state (0.74 ± 0.40 vs. 0.38 ± 0.20 mmol/L; $P = 0.011$) and during hyperinsulinemia (0.55 ± 0.32 vs. 0.17 ± 0.10) mmol/L; $P < 0.001$). The reduction in VLDL-TG concentrations was significant ($P < 0.005$) in both groups (Fig. 3*A* and *G*). However, the relative reduction was impaired in obese men (−22 ± 20 vs. −56 ± 11%; $P < 0.001$).

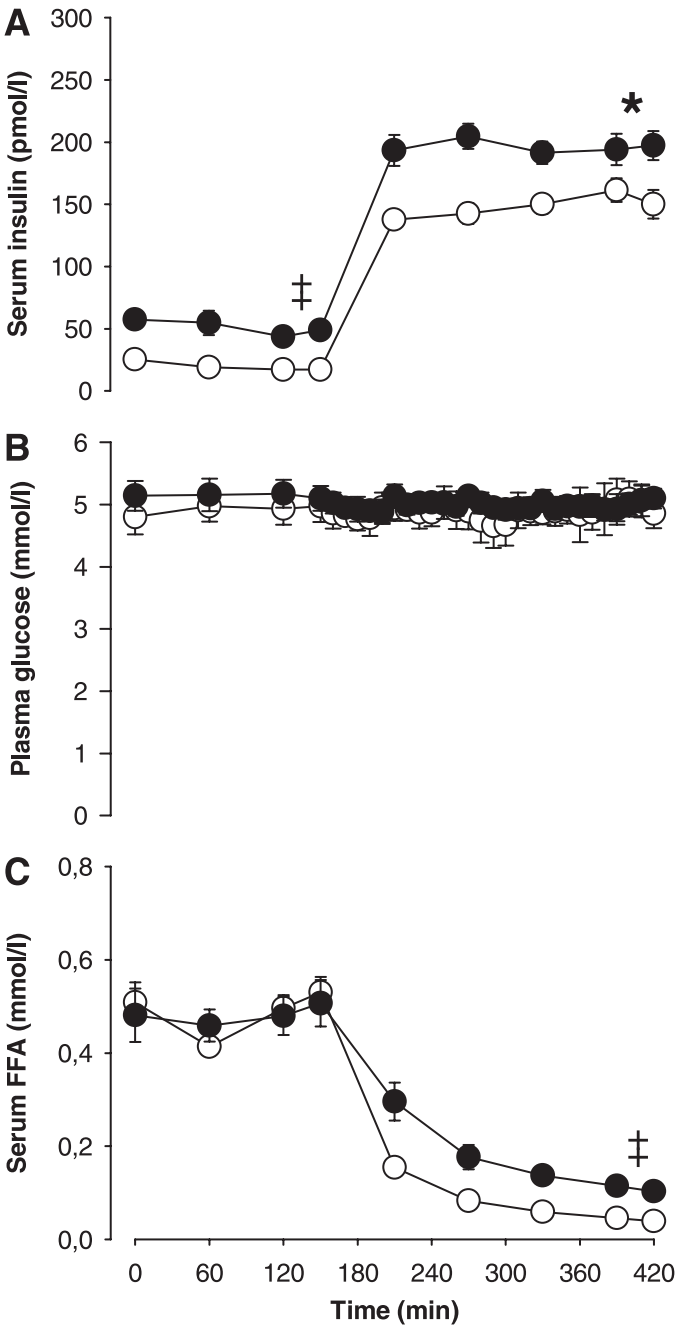

FIG. 2. Concentrations of insulin (*A*), glucose (*B*), and FFAs (*C*) in the basal state and during the hyperinsulinemiceuglycemic clamp. *$P < 0.05$ and ‡$P < 0.001$ between groups in the steady-state periods (120–150 and 390–420 min). Data are presented as means ± SEM. ●, obese men; ○, lean men.

VLDL-TG–to–VLDL-apoB-100 ratio. The basal VLDL–apoB-100 concentration was somewhat greater, although not significantly, in obese men (155 ± 93 vs. 109 ± 71 mg/L; $P = 0.188$) and remained unsuppressed by hyperinsulinemia, whereas in lean men a significant reduction ($P < 0.001$) was noted. The achieved concentration was significantly lower in lean men (143 ± 85 vs. 57 ± 38 mg/L; $P = 0.004$). There was no significant difference in the VLDL-TG–to–VLDL-apoB ratio, a measure of particle size, in obese and lean men in the basal period (5.18 ± 1.57 vs. 3.94 ± 1.57 μmol/mg; $P = 0.067$) or during hyperinsulinemia (4.44 ± 1.68 vs. 3.22 ± 1.06 μmol/mg; $P = 0.053$) (Fig. 3*H*). A slight decrease in the ratio was noted in both groups, but the decrease was significant only in lean men ($P = 0.023$). There was no significant difference between obese and lean men in the absolute (0.72 ± 0.95 vs. 0.74 ± 1.24%) or relative decrease (−14 ± 23 vs. −15 ± 22%; $P = 0.885$).

Glucose kinetics. Corrected for FFM, EGP was lower in obese compared with lean men in the basal period (2.0 [1.7–2.3] vs. 2.3 mg/kg FFM/min [2.1–2.6]; $P < 0.001$), whereas a near-complete suppression was achieved during hyperinsulinemia (0.4 [0.0–0.8] vs. 0.1 mg/kg FFM/min [0.0–1.2]; $P = 0.139$). The reduction in EGP during insulin infusion was significant ($P < 0.001$) in both groups. There was no difference in the relative reduction (80% [57–98] vs. 98% [49–100]; $P = 0.09$), whereas the absolute reduction was greater in lean men (1.7 [1.0–2.0] vs. 2.1 mg/kg FFM/min [1.2–2.4]; $P = 0.006$) (Fig. 4*A* and *B*). The average glucose infusion rate during the clamp steady-state period (*M* value) was lower in obese compared with lean men (4.6 ± 1.3 vs. 9.9 ± 2.2 mg/kg FFM/min; $P < 0.001$) (Fig. 4*C* and *D*). Likewise, glucose R_d was decreased in obese compared with lean men in both the basal state (2.0 ± 0.2 vs. 2.3 ± 0.2 mg/kg FFM/min; $P < 0.001$) and during hyperinsulinemia (4.8 ± 1.0 vs. 10.3 ± 1.6 mg/kg FFM/min; $P < 0.001$). The increase in glucose R_d during hyperinsulinemia was significant ($P < 0.001$) in both groups, but the absolute ($P = 0.001$) and relative (139 ± 57% vs. 348 ± 82%; $P < 0.001$) response (Fig. 4*E* and *F*) was considerably impaired in obese men.

Energy expenditure and substrate oxidation. The respiratory quotient was comparable in the basal period (Table 2), but the relative change in response to hyperinsulinemia was decreased in obese men ($P = 0.010$), reflecting metabolic inflexibility. Accordingly, the relative increase in glucose oxidation and nonoxidative glucose disposal (NOGD) was decreased in obese men, although the difference was only statistically significant for NOGD ($P = 0.157$ and $P = 0.003$, respectively), whereas the relative reduction in lipid oxidation was decreased in obese men ($P = 0.003$).

Correlations. No significant correlations were found between basal VLDL-TG secretion and EGP or serum insulin or between the relative reduction in VLDL-TG secretion and EGP during hyperinsulinemia in either group. In lean men, the *M* value was significantly related to the VLDL secretion rate in the basal state ($r = -0.71$, $P = 0.01$) and during the clamp (−0.62, $P = 0.03$). No significant relationship was found in obese men. No significant relationships were noted between *M* value and VLDL clearance rates.

DISCUSSION

In this study, we assessed the impact of male obesity on basal and insulin-mediated VLDL-TG and glucose kinetics. In addition, we explored whether a coordinated relationship existed between VLDL-TG secretion rate and EGP in insulin-resistant obese men and lean men. We found that basal VLDL-TG secretion rates were significantly increased in obese men compared with lean men, whereas there was no significant difference in VLDL-TG clearance rates, resulting in greater plasma VLDL-TG concentrations. Moreover, although the absolute insulin-mediated suppression of VLDL-TG secretion was similar in the two groups, the percentage change was significantly lower in obese men, and the achieved level during insulin suppression was significantly greater in obese men. Furthermore, although clearance rates decreased in obese men, there was no

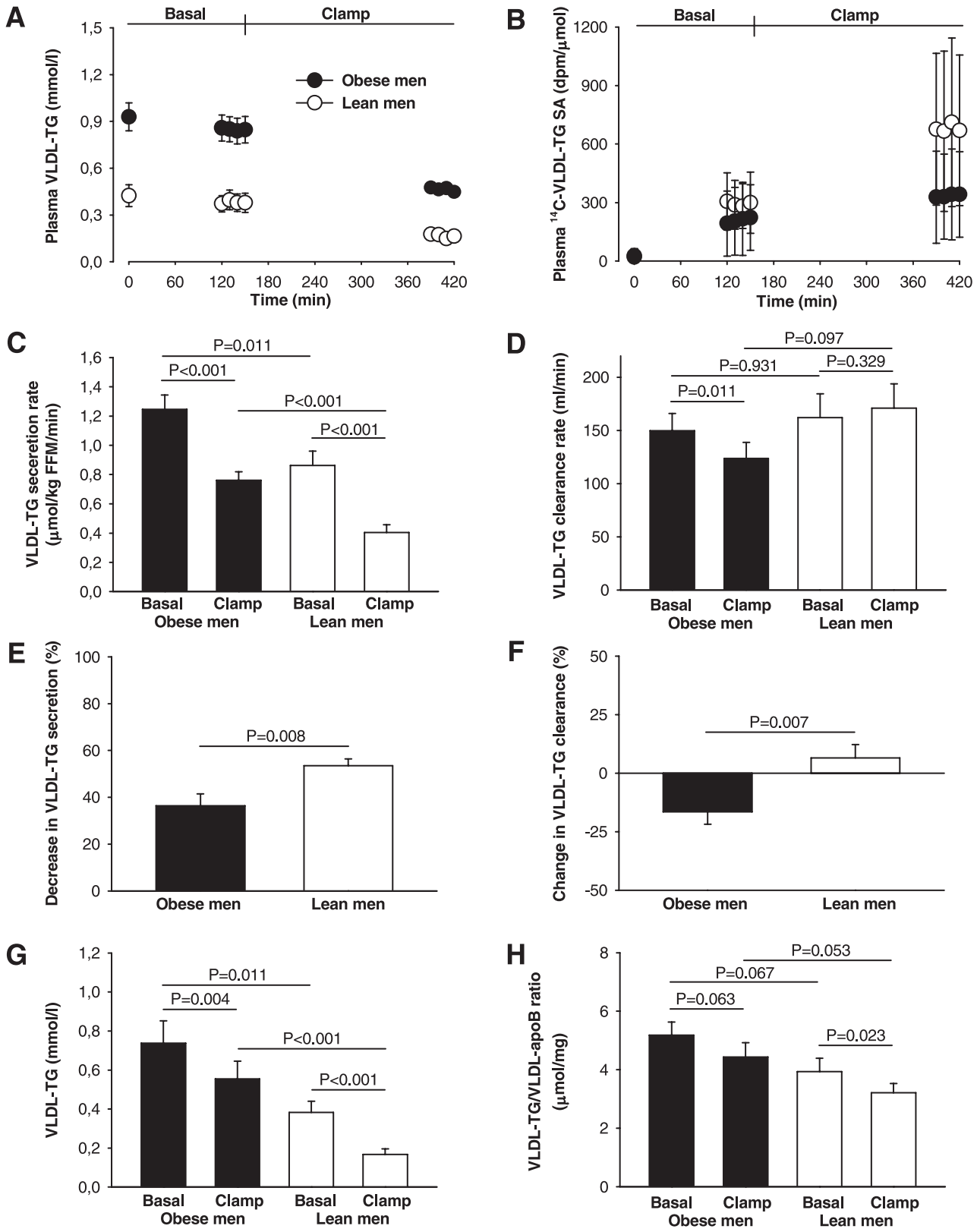

FIG. 3. [^{14}C]VLDL-TG SA (*B*) in the basal and the hyperinsulinemic-euglycemic clamp steady-state periods (120–150 and 390–420 min). VLDL-TG secretion rates (*C*) and VLDL-TG clearance rates (*D*) in the basal and clamp periods and the relative changes in secretion rates (*E*) and clearance rates (*F*) during hyperinsulinemia. VLDL-TG concentrations (*A* and *G*) and the VLDL-TG–to–VLDL-apoB ratio (*H*) in the basal and clamp steady-state periods. Data are presented as means ± SEM. ● and ■, obese men; ○ and □, lean men.

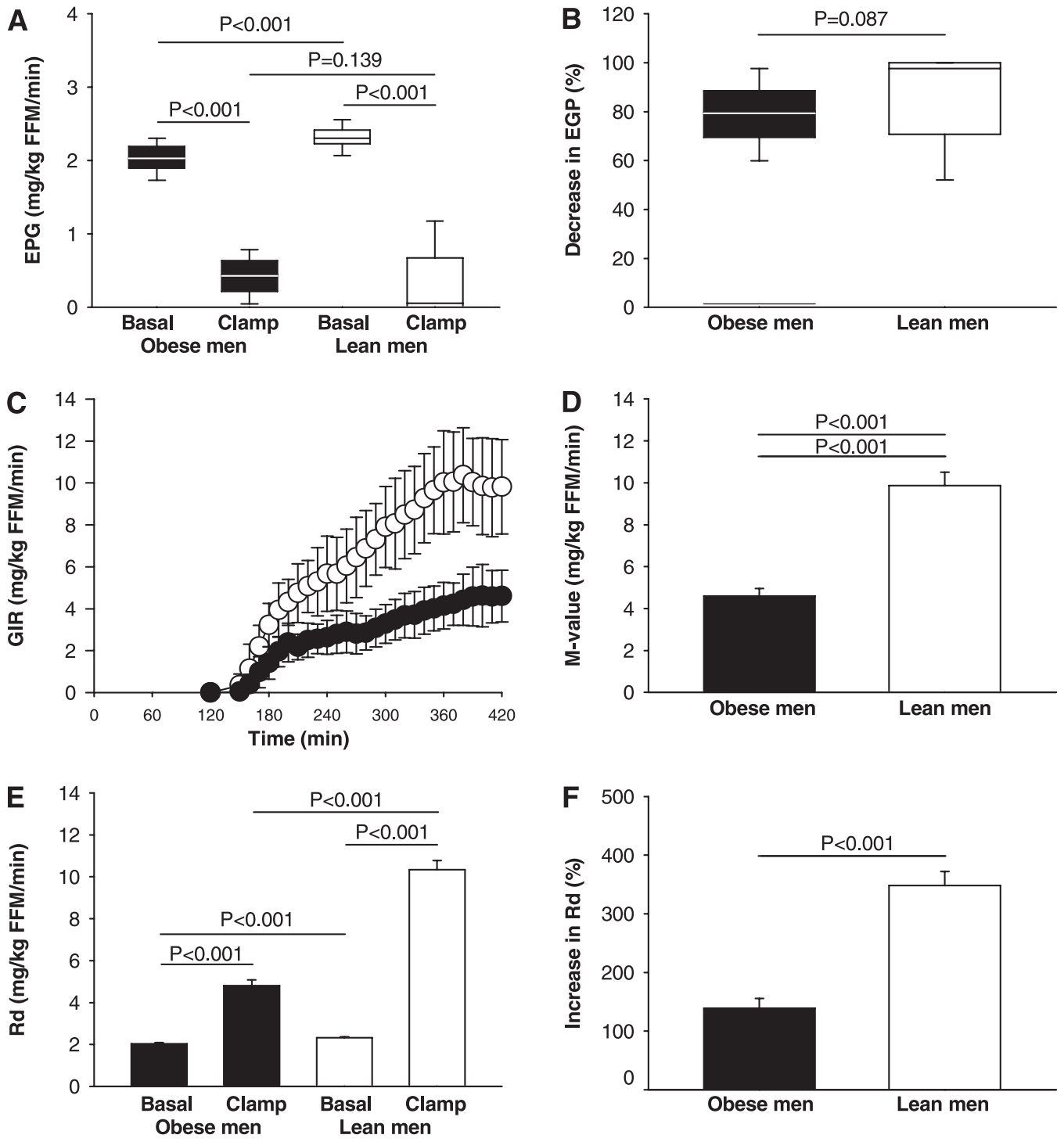

FIG. 4. EGP in the basal and the hyperinsulinemic-euglycemic clamp steady-state periods (120–150 and 390–420 min) (*A*) and the relative changes in EGP during hyperinsulinemia (*B*). Glucose infusion rates (GIR) during the clamp (*C*) and the average GIR during the last 30 min of the clamp (*M* value) (*D*). Glucose rate of disappearance (R_d) in the steady-state periods (*E*) and the relative changes in R_d during hyperinsulinemia (*F*). Data are presented as means ± SEM. ● and ■, obese men; ○ and □, lean men.

significant change in lean men, resulting in less reduction of VLDL-TG concentrations in obese men. On the other hand, we found no indication of impaired suppression of EGP in obese men despite significant insulin resistance with respect to glucose disposal.

The impact of male obesity on basal VLDL-TG and VLDL-apoB kinetics has been examined in relatively few studies, all on the basis of in vivo labeling and mathematical modeling (7–10). As in the current study, Mittendorfer et al. report greater secretion of VLDL-TGin obese men than in lean men. ApoB kinetic studies by Chan et al. have extended these findings by demonstrating that apoB production rates (a measure of VLDL particle secretion rate) are greater in obese subjects than in lean subjects. Taken together with our observation that the apoB-to-TG ratio, and thus VLDL particle size, was comparable in lean and

TABLE 2
Energy expenditure, respiratory quotient, and substrate oxidation rates

	Obese men			Lean men		
	Basal	Clamp	*P*	Basal	Clamp	*P*
Energy expenditure (kcal/day)	2,112 ± 182‡	2,074 ± 150†§	0.196	1,790 ± 215	1,849 ± 209	0.003
Respiratory quotient	0.81 ± 0.03	0.86 ± 0.04‡§	<0.001	0.83 ± 0.04	0.92 ± 0.04	<0.001
Glucose oxidation (mg/kg FFM/min)	1.20 ± 0.47*	2.00 ± 0.62‡	<0.001	1.68 ± 0.59	3.54 ± 0.72	<0.001
NOGD (mg/kg FFM/min)	0.83 ± 0.44	2.80 ± 0.78‡‖	<0.001	0.64 ± 0.60	6.80 ± 1.75	<0.001
Lipid oxidation (mg/kg FFM/min)	1.05 ± 0.29	0.70 ± 0.26†‖	<0.001	1.01 ± 0.30	0.35 ± 0.31	<0.001

Data are means ± SD or median (range). *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ vs. lean men. §$P < 0.05$; ‖$P < 0.01$; ¶$P < 0.001$ relative difference (basal clamp) versus relative difference (basal clamp) in lean men.

obese men, these findings indicate that more VLDL particles, not larger ones, are secreted from the liver in obese subjects compared with lean subjects. Our study differs from the one by Mittendorfer et al. on one point, however; we report substantially greater absolute secretion rates of VLDL-TG (micromoles per minute). This discrepancy may relate to differences in tracer method (glycerol tracer bolus and modeling versus steady-state tracer technique) and in body composition. Methods based on in vivo labeling and mathematical modeling rely on a single-pool model, and VLDL-TG secretion rates are calculated by multiplying VLDL-TG pool size by FCR (19). However, we recently reported that VLDL-TG kinetics are better described by a two-pool model, meaning that FCR and VLDL-TG turnover are underestimated assuming single-pool kinetics (20). Moreover, the FFM of our obese study subjects was greater than those reported by Mittendorfer et al. Because we recently found that REE and FFM are significant independent predictors of VLDL-TG turnover (21), at least some of the difference, may be explained by differences in study group phenotype.

How insulin regulates VLDL-TG production has been the focus of recent studies prompted by the observation that insulin-resistant individuals are hypertriglyceridemic. Cell and rodent studies have demonstrated that insulin in the hepatocyte acts via the insulin receptor and insulin receptor substrate-2 to activate downstream signaling (phosphoinositide 3-kinase and protein kinase B), ultimately dislocating FoxO1 from the nucleus of the cell. Because FoxO1 augments MTP activity, a key enzyme facilitating the uptake of TG droplets into the VLDL particle (22), insulin effectively acts as a brake on VLDL-TG secretion. Therefore, the inability of insulin to check the activity of FoxO1 may result in VLDL overproduction because it is observed in insulin-resistant individuals. Impaired inactivation of FoxO1 also contributes to glucose-mediated increases in VLDL production, as demonstrated by Wu et al. (23). However, insulin not only serves as a brake on VLDL-TG output, it may itself promote hepatic TG production by upregulating lipogenic enzymes, such as sterol regulatory element–binding protein-1c, leading to de novo lipogenesis. This lipogenic effect of insulin may be mediated via activation of insulin receptor substrate-1 and is presumably not lost in insulin-resistant states (24,25). Taken together, these studies indicate that the effect of insulin on VLDL-TG secretion depends on whether concentrations are raised acutely, leading to inhibition of VLDL-TG secretion or rise more gradually, leading to upregulation of lipogenic enzymes, de novo lipogenesis, and, ultimately, VLDL-TG secretion. In vivo studies of VLDL-TG kinetics tend to support this notion. Thus, experimental hyperinsulinemia has been shown to decrease VLDL-TG (11) and VLDL-apoB (11–13). In the semiquantitative study by Lewis et al., hyperinsulinemia exerted a greater effect on VLDL-TG than on VLDL-apoB, and in the studies by Malmström et al., insulin infusion suppressed $VLDL_1$-apoB secretion with only little effect on $VLDL_2$-apoB secretion. Furthermore, the effect of insulin infusion on VLDL kinetics has been compared in subjects with high liver fat (80% with type 2 diabetes) and low liver fat (all nondiabetic) (26). Hyperinsulinemia resulted in a rapid decline in $VLDL_1$-TG and VLDL-apoB secretion in the group with low liver fat, whereas there was no significant change in the $VLDL_1$ secretion in the group with high liver fat. Moreover, Lewis et al. (11) demonstrated that hyperinsulinemia still suppresses VLDL-TG secretion even during simultaneous lipid infusion to stabilize FFA levels, a substrate for VLDL-TG synthesis. In a recent study of type 2 diabetic and age- and BMI-matched healthy men using a design comparable to the current study, except for a greater insulin infusion rate (1.0 μU/kg FFM/min) (27), we reported that VLDL-TG secretion was significantly suppressed by insulin in both groups. Although we observed no significant difference in the relative suppression of VLDL-TG secretion rates, the relative suppression of VLDL-TG concentration and the VLDL-TG–to–VLDL-apoB ratio was significantly impaired in type 2 diabetic men. Of interest, the reduction in VLDL-TG–to–VLDL-apoB ratio was lower in the current study compared with the study including type 2 diabetic men and healthy men, suggesting that the reduction in VLDL particle TG content in response to hyperinsulinemia is regulated dose dependently. However, other factors could contribute to the difference, such as greater insulin resistance in the obese men in the current study compared with the obese control group in our previous publication, as suggested by greater body weight, fasting serum insulin, and VLDL-TG concentrations. The notion that long-term hyperinsulinemia (and hyperglycemia) promotes rather than inhibits VLDL-TG output has been demonstrated in humans by Aarsland et al. (28). We also reported insulin concentrations to be an independent predictor of VLDL-TG secretion in a cross-sectional study (21).

One aim was to explore whether insulin inhibits VLDL-TG secretion and EGP to a similar extent in both lean and obese individuals. We found this interesting because postprandial insulin-mediated suppression of VLDL-TGs and glucose secretion is appropriate to facilitate clearance of excess nutrients from circulation. To our knowledge, this relationship has not been previously evaluated in human studies. We used gold-standard methods; the hyperinsulinemic-euglycemic clamp technique was applied to study the isolated effect of moderate hyperinsulinemia, and glucose production and use was determined using [3-^{3}H]glucose tracers in combination with indirect calorimetry. As a novel observation, we found that insulin-mediated

suppression of VLDL-TG secretion was impaired in obese men, whereas, at the same time, the suppression of EGP was preserved in both lean and obese subjects. Although the absolute reduction in VLDL-TG secretion was similar in the two groups, both the percentage change as well as the achieved secretion rate were significantly less pronounced in obese men compared with lean men. The obese participants therefore seemed to be somewhat insulin resistant with respect to VLDL-TG secretion but not maintenance of normoglycemia. It has been suggested (29) that incomplete global loss of insulin signaling may impact more severely upon some arms of a signaling network than others, according to their different patterns of ligand dose responsiveness. This may explain why insulin-resistant individuals tend to be unable to downregulate VLDL-TG output under conditions of hyperinsulinemia (as a result of the loss of the inhibitory effect) but still have substantial de novo lipogenesis. The concept of incomplete insulin resistance also could explain why our obese and peripherally insulin-resistant volunteers maintained the inhibitory effect of insulin on EGP while being unable to inhibit VLDL-TG secretion to the same extent as lean men. In accordance with this, despite similar FFA concentrations in the two groups, it is likely that obese men were subjected to greater hepatic FFA delivery from visceral fat lipolysis than lean men, which, in addition to yet-unexplored genetic differences, could contribute to the increased VLDL-TG secretion.

The study may have methodological limitations. The VLDL-TG tracer was prepared from plasma obtained in the postabsorptive state, and because VLDL particle composition changes in response to hyperinsulinemia, the VLDL-TG tracer used to determine VLDL-TG kinetics during hyperinsulinemia should ideally have been prepared from plasma obtained during hyperinsulinemia. However, Lewis et al. (30) found no differences in VLDL-apoB kinetics whether plasma was drawn under basal or hyperinsulinemic conditions. Moreover, the relatively low number of subjects could introduce type 2 errors in some statistical comparisons. Regarding VLDL secretion, however, we previously found significant reductions during hyperinsulinemia in obese and type 2 diabetic men.

In summary, compared with lean men, normoglycemic, but peripherally insulin-resistant, obese men have *1*) greater basal VLDL-TG secretion and similar VLDL-TG clearance rates resulting in increased VLDL-TG concentrations, *2*) impaired ability to achieve suppression of VLDLTG secretion to a level similar to control subjects under hyperinsulinemia, and *3*) preserved blocking of EGP. Our data indicate that obesity-related insulin resistance may affect VLDL-TG production before gluconeogenesis and glycogenolysis.

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

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