Sensitivity to Acute Insulin-Mediated Suppression of Plasma Free Fatty Acids Is Not a Determinant of Fasting VLDL Triglyceride Secretion in Healthy Humans

André Carpentier,[1] Bruce W. Patterson,[2] Nathalie Leung,[1] and Gary F. Lewis[1]

One important mechanism whereby obesity-associated insulin resistance leads to VLDL overproduction is thought to be by the increased flux of free fatty acids (FFAs) from extrahepatic tissues to liver, which arises as a direct consequence of impaired insulin action in adipose tissue and skeletal muscle. The aim of the present study was to address whether direct measures of peripheral tissue insulin sensitivity with regard to FFAs and glucose in the fasting state are good predictors of postabsorptive VLDL triglyceride secretion rate (VLDL-TG ASR) in humans, independent of obesity. Eighteen healthy control subjects, after an overnight fast, underwent three studies 3 weeks apart, in random order. Study 1: VLDL-TG levels, fractional clearance rate (per h), and VLDL-TG ASR were determined after an intravenous bolus of [1,1,2,3,3-$^2H_5$]glycerol. Study 2: Insulin sensitivity (S_I), acute insulin response (AIR), and acute C-peptide response to glucose were assessed by frequently sampled intravenous glucose tolerance test using the minimal model approach. Study 3: Insulin-mediated suppression of plasma FFAs (k) and insulin clearance were assessed in response to a low-dose stepwise intravenous insulin infusion. BMI ($R^2 = 0.54$), AIR, and fasting insulin levels were positively and S_I negatively correlated with VLDL-TG ASR, but there was no significant association with plasma FFAs or k. Only BMI remained significantly associated with VLDL-TG ASR in multivariate analysis. The best multivariate model for VLDL-TG ASR ($R^2 = 0.61$, $P = 0.0008$) included BMI ($P = 0.0008$) and S_I ($P = 0.12$, inversely correlated). VLDL-TG secretion is predicted by BMI, independently of direct measures of insulin sensitivity. The sensitivity to insulin's acute suppressive effect on plasma FFA levels during fasting is not an important determinant of postabsorptive VLDL-TG secretion in humans. *Diabetes* 51:1867–1875, 2002

From the [1]Department of Medicine, Division of Endocrinology, University of Toronto, and the Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada; and the [2]Department of Internal Medicine and Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to Dr. Gary Lewis, The Toronto General Hospital, 200 Elizabeth St., Room EN 11-229, Toronto, ON, Canada M5G 2C4. E-mail: gary.lewis@uhn.on.ca.

Received for publication 28 June 2001 and accepted in revised form 27 February 2002.

ACR, acute C-peptide response; AIR, acute insulin response; apoB, apolipoprotein B; Cl_{ins}, plasma insulin clearance rate; CV, coefficient of variation; FCR, fractional clearance rate; FFA, free fatty acid; GC/MS, gas chromatography/mass spectrometry; ISR, insulin secretion rate; IV, intravenous; k, insulin-mediated suppression of plasma FFAs; mFSIVGTT, insulin-modified frequently-sampled intravenous glucose tolerance test; R_a, insulin appearance rate; S_I, insulin sensitivity index; TG, triglyceride; VLDL-TG ASR, VLDL-TG secretion rate.

Obesity, particularly when excess body fat is predominantly abdominal in distribution, is associated with resistance to insulin action (1). Insulin resistance underlies type 2 diabetes and a high proportion of atherosclerotic cardiovascular disease, both of which are occurring in epidemic proportions in developed and developing countries (2,3). Epidemiological studies have shown that insulin resistance is associated with a triad of lipid abnormalities, including hypertriglyceridemia, low HDL cholesterol levels, and small, dense LDL particles (4–6). This metabolic phenotype is of great clinical importance, as it is thought to be responsible for a large part of the increased cardiovascular risk associated with the insulin resistance syndrome and type 2 diabetes (7,8).

Lipid turnover studies in humans and animal models of insulin resistance, obesity, and type 2 diabetes have shown that the hypertriglyceridemia associated with these conditions is due predominantly to elevated VLDL production (9). The precise mechanisms linking obesity and insulin resistance to VLDL hypersecretion, however, are still debated. Insulin plays a pivotal role in controlling the production rate of VLDL in both a direct and an indirect fashion. VLDL secretion is largely a substrate-driven process, and insulin plays a pivotal role in controlling the flux of biosynthetic precursors such as free fatty acids (FFAs) from extrahepatic tissues to the liver (10), in addition to controlling multiple steps in hepatic VLDL assembly and secretion (11). Peripheral tissue insulin sensitivity, particularly in adipose tissue and skeletal muscle, is therefore believed to be closely linked to VLDL production via insulin's important role in extrahepatic triglyceride (TG) storage and its mobilization in the form of FFAs and glycerol (12). It is generally accepted, therefore, that one important mechanism whereby obesity-associated insulin resistance leads to VLDL overproduction is the increased flux of FFAs from extrahepatic tissues to liver, which arises as a direct consequence of impaired insulin action in adipose tissue and skeletal muscle (13). The close metabolic and genetic links between the various clinical features of the insulin resistance syndrome (such as obesity, hypertriglyceridemia, and insulin resistance), however, preclude a careful assessment of the mechanistic relationship between these features in large epidemiological studies, which have generally measured indirect or surrogate markers of TG production and insulin resistance.

This is the first study to investigate whether direct measures of insulin sensitivity are truly predictive of hepatic VLDL-TG production rate, independent of obesity. We assessed two major aspects of peripheral tissue insulin sensitivity in vivo, insulin-mediated glucose uptake and insulin-mediated suppression of plasma FFAs, in addition to measures of insulin secretion, and correlated these with BMI, waist circumference, and a measure of VLDL-TG production in each of 18 healthy individuals. We found that, contrary to what is commonly assumed, the sensitivity of insulin-mediated suppression of fasting plasma FFAs showed no correlation whatsoever with postabsorptive VLDL production rate. BMI was by far the strongest predictor, and insulin-mediated glucose disposal was a weak predictor of postabsorptive VLDL production rate.

RESEARCH DESIGN AND METHODS

Subjects. Eighteen healthy subjects (10 women, 8 men), aged 18–60 years with a BMI between 18 and 35 kg/m^2, participated in the studies. None were diabetic based on repeated assessment of fasting glucose concentration (14). One subject had mildly impaired fasting blood glucose. None of the study participants were taking any medication, had any current medical condition known to affect lipid levels or insulin sensitivity, had any known cardiovascular disease, or had any surgical intervention within 6 months before the studies. All the women who participated were premenopausal, and the studies were conducted during the follicular phase of their menstrual cycle. Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of the University Health Network.

Experimental protocols. All of the subjects participated in three studies (as described below) 3–4 weeks apart, in random order. They were on a stable diet, had no change in their degree of physical activity, and had stable weight for at least 3 months before and throughout the study period. All studies were performed in the Metabolic Investigation Unit of the Toronto General Hospital, where the patients were admitted on each occasion between 7:30 and 8:30 A.M., following a 12-h overnight fast. They remained fasting during the studies, but had access to water ad libitum. For each study, an intravenous catheter was placed in each forearm, one for infusion and one for blood sampling. The arm containing the sampling catheter was maintained in a heating blanket (~65°C) to arterialize venous blood. Body weight and height were assessed during fasting on the morning of the three studies; waist circumference was measured at midlevel between the costal and iliac ridge while standing.

Study 1: VLDL-TG turnover. All of the study participants received detailed instructions to maintain an isocaloric diet containing 30% fat, 20% protein, and 50% carbohydrate for 2 days before these studies. After a 12-h overnight fast, the participants received an intravenous bolus injection of [1,1,2,3,3-$^2H_5$]glycerol (100 μmol/kg) (Cambridge Isotopes Laboratories, Andover, MA) to determine VLDL-TG kinetics, as previously described (15). The tracer was dissolved in sterile normal saline and was tested for sterility and pyrogenicity before injection. Blood samples were taken at 0, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, 420, 600, 660, and 720 min for separation of the VLDL fraction (Svedberg flotation 20-400) by ultracentrifugation and determination of VLDL-TG [1,1,2,3,3-$^2H_5$]glycerol enrichment. The samples were collected into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing Na_2EDTA and placed immediately on ice, and the plasma was separated within 2 hours. VLDL apolipoprotein B (apoB) and VLDL-TG concentrations were also measured at 0, 120, 240, 360, 600, and 720 min to verify that a steady-state level was maintained. The averages of these measurements were taken as the VLDL-apoB and VLDL-TG levels.

Study 2: insulin-modified frequently-sampled intravenous glucose tolerance test. An insulin-modified frequently-sampled intravenous glucose tolerance test (mFSIVGTT) was performed after a 12-h overnight fast to determine the insulin sensitivity index (S_I), using the minimal model approach (16,17). Briefly, after three baseline blood samples, an IV bolus of glucose (0.3 g/kg; dextrose 50%) was administered at time 0, and an IV bolus of biosynthetic human insulin (0.03 U/kg) (Humulin R; Eli Lilly, Toronto, Canada) was injected at 20 min. Blood samples were taken at 2, 3, 4, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min for determination of plasma glucose, insulin, and C-peptide levels.

Study 3: graded intravenous insulin infusion. This protocol is a modification of our previously published protocol (18) that allows the determination of insulin-mediated suppression of fasting plasma FFAs while keeping constant the plasma glucose level. Essentially, this protocol was designed to provide a single integrated measure of the suppressibility of plasma FFA concentrations in response to a low-dose, stepwise increment in plasma insulin concentration during euglycemia. This protocol also allowed us to calculate insulin clearance for each individual (see below). After a 30-min baseline period, a programmed IV insulin (Humulin R; Eli Lilly) infusion was started at a rate of 1 mU · m^{-2} · min^{-1} and was increased in a stepwise fashion to 2, 4, 8, 16, and 32 mU · m^{-2} · min^{-1} every 40 min. An IV glucose infusion (dextrose 20%), adjusted every 5 min according to the plasma glucose level, was used to maintain plasma glucose at the fasting level. Glucose levels did not change from baseline ($P > 0.20$) (not shown). This protocol resulted in small stepwise increments of plasma insulin levels from 0 to ~35 mU/l above basal levels, which allowed for enough discrimination of the suppression of plasma FFAs by insulin in healthy subjects (19). Blood samples were taken at 10-min intervals throughout the study for measurement of glucose, insulin, C-peptide, and FFAs. Blood samples were collected on ice into tubes containing Na_2EDTA and 30 μg of the lipase inhibitor tetrahydrolipstatin (Orlistat; Hoffman La Roche, Mississauga, ON, Canada) per milliliter blood to prevent ongoing in vitro lipolysis of the samples (20). Figure 1 shows insulin infusion rate (*A*), plasma glucose (*B*), insulin (*C*), FFAs (*D*), insulin appearance rate versus change in insulin (*E*) (used to calculate insulin clearance; see below), and FFAs versus change in insulin (*F*) (used to calculate insulin-mediated suppression of plasma FFAs, *k*; see below) in a representative subject.

Laboratory methods. Glucose was assayed enzymatically at the bedside using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay using a double-antibody separation method (kit supplied by Pharmacia Diagnostic, Uppsala, Sweden) (intra- and interassay coefficients of variation [CVs], 5.8 and 11.2%, respectively). C-peptide was measured by a double-antibody C-peptide radioimmunoassay (kit supplied by Diagnostic Products Corporation, Los Angeles) (intra- and interassay CVs, 4.1 and 15.1%, respectively). FFAs were measured by a colorimetric method (kit supplied by Wako Industrials, Osaka, Japan) (CV <3%). Cholesterol and TG were measured for each lipoprotein fraction using colorimetric assays (Boehringer Mannheim GmbH Diagnostica) (CV <3%). ApoB was measured in each fraction using an electroimmunoassay, as previously described (21) (CV <10%). VLDL was isolated from the plasma by ultracentrifugation at density 1.006 g/ml using a type 50.3 Ti rotor (Ultracentrifuge model L870; Beckman, Palo Alto, CA) at 39,000 rpm and 16°C for 16 h.

The VLDL fraction was delipidated with diethyl ether/methanol in acid-washed glass tubes to separate VLDL lipids from VLDL proteins. The supernatant containing VLDL lipids (TG, free cholesterol, cholesteryl ester, and phospholipids) was separated and dried under nitrogen and VLDL-TG and then isolated by thin-layer chromatography on 20 × 20 cm silica gel plates (06-600A Gel G TLC; Fisher) using heptane:isopropyl ether:acetic acid 80:20:2 (22). VLDL-TG glycerol was recovered and converted to heptafluorobutyryl derivative as previously described (15), and enrichment of [1,1,2,3,3-$^2H_5$]glycerol was measured by electron impact ionization gas chromatography/mass spectrometry (GC/MS) using a 30 m × 0.25 mm, 0.25 m film DB-17 capillary column (Supelco) on a model 5973 quadrupole GC/MS (Hewlett-Packard). Glycerol (m/z 467) and its m+5 isotopomer (m/z 472) were measured, and the glycerol tracer:tracee ratio was determined by calibration of measured m+5/m+0 ratios for standards of known isotopic enrichment (CV <2%).

Calculations

VLDL-TG kinetics. The fractional clearance rate (FCR) of VLDL-TG was estimated from the monoexponential slope of VLDL-TG [$^2H_5$]glycerol isotopic enrichment immediately after the maximum enrichment, as previously described (15). We have recently shown that the monoexponential slope captures the major feature of the VLDL-TG time course curve, which is the turnover of VLDL-TG (tracer recycling is a minor feature) and is an index that correlates nonlinearly with the FCR (23). The VLDL-apoB and VLDL-TG levels did not change significantly over time during the 12-h turnover study ($P > 0.20$); therefore, steady state was assumed. VLDL-TG secretion rates (VLDL-TG ASR) were calculated as follows (15):

$$\text{VLDL-TG ASR} = [\text{VLDL-TG}] \times \text{FCR (per min)} \times 0.039\ (\text{l/kg}) \times 24\ (\text{h})/\text{body wt (kg)}$$

Where [VLDL-TG] is the mean concentration of VLDL-TG (in millimoles per liter) over the entire 12 h of the study.

Insulin sensitivity index, acute insulin response, and acute C-peptide response to IV glucose. S_I (10^4 · min^{-1} · μU^{-1} · ml^{-1}) was calculated using MINMOD software (version 3.0; provided by Dr. R.N. Bergman) (24). The acute insulin response (AIR) and acute C-peptide response (ACR) were determined as surrogates of the acute glucose-stimulated insulin secretory response by calculating the average of the incremental response from baseline

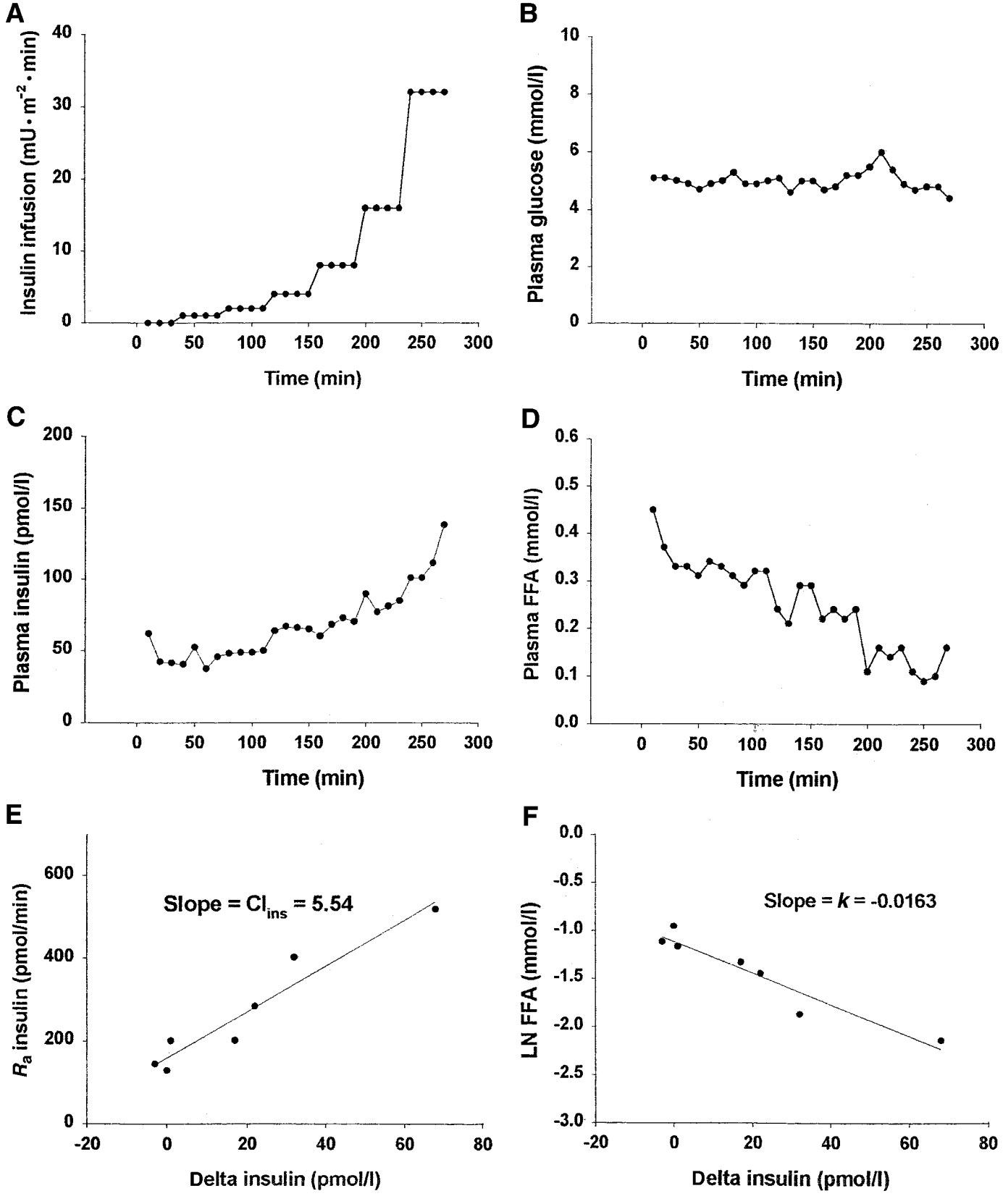

FIG. 1. Data of the graded intravenous insulin infusion study from a representative patient. *A*: Exogenous insulin infusion rate. *B*: Plasma glucose levels. *C*: Plasma insulin levels. *D*: Plasma FFA levels. *E*: Insulin appearance rate (R_a) at the end of each insulin infusion period versus change in insulin from baseline (delta insulin). The slope of the relationship equals insulin clearance (Cl_{ins}). *F*: Natural logarithm of plasma FFAs (LN FFA) at the end of each insulin infusion period versus change in plasma insulin from baseline (delta insulin). The absolute value of the slope of this relationship was used as an index (k) of insulin-mediated suppression of plasma FFAs.

TABLE 1
Clinical characteristics and fasting metabolite and hormone levels of the study participants

	Means ± SE	Range
Age (year)	29 ± 3	20–60
Sex (M/F)	8/10	—
BMI (kg/m^2)	24.2 ± 1.0	18.4–34.8
Waist circumference (cm)	79.6 ± 2.5	70.0–112.5
Fasting plasma glucose (mmol/l)	5.4 ± 0.1	4.6–6.2
Fasting plasma insulin (pmol/l)	49 ± 9	13–177
Fasting plasma C-peptide (nmol/l)	0.37 ± 0.05	0.14–1.04
Fasting plasma FFAs (mmol/l)	0.493 ± 0.036	0.268–0.888
Fasting plasma TG (mmol/l)	1.13 ± 0.18	0.48–3.24
Fasting plasma apoB (mg/dl)	88 ± 4	57–135
VLDL-TG (mmol/l)	0.67 ± 0.16	0.20–2.97
VLDL-apoB (mg/dl)	10.8 ± 1.3	4.5–25.3

of plasma insulin and C-peptide, respectively, at 2, 3, 4, and 5 min after the IV glucose bolus (25).

Plasma insulin clearance rate. To calculate the plasma insulin clearance rate (Cl_{ins}), the endogenous insulin secretion rate (ISR) during the graded insulin infusion study was first assessed by deconvolution of plasma C-peptide (26) using standard kinetic parameters for C-peptide clearance, as previously validated by others (27). ISR during the last 20 min of each insulin infusion step was then added to the exogenous insulin infusion rate to obtain the total insulin appearance rate (R_a) for each insulin infusion step. R_a was plotted against the average insulin levels during the last 20 min of each insulin infusion step, and Cl_{ins} was obtained by determining the slope of the relationship by linear regression (Fig. 1*E*). The average R^2 of the fit was 0.87 ± 0.05, suggesting a good fit of the data.

Rate of decline of plasma FFAs in response to change in plasma insulin during the graded intravenous insulin infusion study. The natural logarithms of the mean plasma FFA levels during the last 20 min of each graded intravenous insulin infusion were plotted against the corresponding change from baseline of plasma insulin, and the data were fitted by linear regression for each subject (Fig. 1*F*). The absolute value of the slope of this relationship, k [natural log(millimoles of FFA suppression)/picomoles of increase in plasma insulin], is proportional to the suppression of plasma FFA levels by insulin over small, stepwise elevations of plasma insulin concentration. The mean R^2 of the fit was 0.80 ± 0.05, suggesting a good fit of the data.

Statistical analysis. The data are expressed as means ± SE. When not normally distributed, the variables were transformed using the mathematical function leading to the best normalization of the data before linear regression analysis. Linear regression analyses were performed with total TG levels, VLDL-apoB levels, VLDL-TG levels, and VLDL-TG kinetic parameters as independent variables and the other metabolic, clinical, and anthropometric variables as dependent variables. For variables available from more than one of the studies (BMI, waist circumference, and fasting glucose, insulin, C-peptide, FFA, and total plasma TG levels), the average values from the three studies are reported and were used for the analyses. Forward-step multivariate linear regression analyses including only variables with at least a trend toward an association ($P < 0.20$) in bivariate analyses were performed to define the best model (two of the following three criteria: maximum adjusted R^2, minimum residual sum of squares, and optimal Mallows C_p) to predict total TG levels, VLDL-TG levels, total plasma apoB levels, VLDL-apoB levels, and VLDL-TG kinetic parameters. For all the analyses, a P value <0.05 was considered significant. All the analyses were performed with SAS software for Windows (version 7; SAS Institute, Cary, NC).

RESULTS

The clinical characteristics and the fasting metabolite and hormone levels of the 18 participants are shown in Table 1. VLDL-TG kinetic parameters and insulin secretion, clearance, and sensitivity parameters are shown in Table 2. Plasma TG, VLDL-TG, and VLDL-apoB levels did not change significantly over time during the VLDL stable isotope administration study and were therefore in steady state ($P > 0.98$).

Determinants of total plasma TG, total plasma apoB, VLDL-TG, and VLDL-apoB fasting levels. Table 3 shows the bivariate correlation between both total TG levels and VLDL-TG levels (log transformed) and the other metabolic and clinical variables. The best multivariate model to predict total TG levels included BMI (log) and Cl_{ins} (square root) ($R^2 = 0.73$, $P < 0.0001$; $P = 0.005$ and $P = 0.03$, respectively). No other variable independently predicted total TG levels in our subjects, and BMI remained independently associated despite the addition of any other variable in the model. The best multivariate model to predict VLDL-TG levels included BMI (log), fasting C-peptide levels, and S_I ($R^2 = 0.76$, $P = 0.0001$; $P = 0.001$, $P = 0.05$, and $P = 0.17$, respectively). BMI was the only variable that remained significantly correlated with VLDL-TG levels when any other variable was included in the model.

Table 4 shows the bivariate correlation between both total apoB levels and VLDL-apoB levels (log transformed) and the other metabolic and clinical variables. The best multivariate model to predict total plasma apoB levels included BMI, age (1/age), and ACR ($R^2 = 0.56$, $P = 0.008$; $P = 0.05$, $P = 0.11$, and $P = 0.21$, respectively). BMI (log) was again the only variable that remained independently correlated with total apoB levels irrespective of the inclusion of any other variable in the model. The best multivariate model to predict VLDL-apoB levels included BMI (log), Cl_{ins} (square root), and age (1/age) ($R^2 = 0.63$, $P = 0.002$; $P = 0.11$, $P = 0.13$, and $P = 0.10$, respectively).

In this healthy population, VLDL-TG ASR (log) and

TABLE 2
VLDL-TG kinetic and insulin secretion, clearance, and sensitivity parameters

	Means ± SE	Range
VLDL-TG kinetics		
FCR (h^{-1})	0.48 ± 0.04	0.15–0.98
VLDL-TG-ASR (mmol/d)	18.1 ± 3.2	5.7–52.3
Insulin secretion		
AIR (pmol/l)	286 ± 54	44–878
ACR (nmol/l)	0.72 ± 0.12	0.03–1.98
Insulin clearance (l/min)	2.46 ± 0.38	0.04–6.68
S_I ($\times 10^{-4} \cdot min^{-1} \cdot mU^{-1} \cdot l^{-1}$	4.3 ± 0.6	0.7–9.7
k [log (mmol ΔFFA/pmol Δinsulin)]	0.021 ± 0.004	0.001–0.067

TABLE 3
Linear regression analyses between total TG and VLDL-TG levels and clinical, anthropometric, and metabolic dependent variables

	Log (total plasma TG)		Log (VLDL-TG)	
	R^2	P	R^2	P
1/Age	0.13	0.13	0.11	0.17
Sex (female)	0.05	>0.20	0.04	>0.20
Log (BMI)	0.63	0.0001	0.65	<0.0001
1/Waist circumference	0.16	0.16	0.17	0.14
Fasting glucose	0.16	0.10	0.14	0.13
Log (fasting insulin)	0.17	0.09	0.31	0.02
Fasting C-peptide	0.21	0.05	0.42	0.004
Fasting FFAs	<0.01	>0.20	0.08	>0.20
Log (AIR)	0.20	0.06	0.09	0.13
ACR	0.02	>0.20	0.09	>0.20
S_I	0.14	0.13	0.11	0.19
Square root of Cl_{ins}	0.54	0.0005	0.37	0.0076
Square root of k	0.14	0.12	0.08	>0.20

TABLE 4
Linear regression analyses between total plasma apoB and VLDL-apoB levels and clinical, anthropometric, and metabolic dependent variables

	Total plasma apoB		Log (VLDL-apoB)	
	R^2	P	R^2	P
1/Age	0.30	0.02	0.27	0.03
Sex (female)	<0.01	>0.20	0.03	>0.20
Log (BMI)	0.41	0.004	0.50	0.001
1/Waist circumference	0.13	>0.20	0.12	>0.20
Fasting glucose	0.05	>0.20	0.08	>0.20
Log (fasting insulin)	0.13	0.15	0.15	0.11
Fasting C-peptide	0.23	0.04	0.33	0.01
Fasting FFAs	<0.01	>0.20	<0.01	>0.20
Log (AIR)	0.03	>0.20	0.12	0.15
ACR	0.16	0.10	0.04	>0.20
S_I	0.04	>0.20	0.15	0.11
Square root of Cl_{ins}	0.27	0.03	0.36	0.008
Square root of k	0.07	>0.20	0.09	>0.20

TABLE 5
Linear regression analyses between VLDL-TG FCR and ASR and clinical, anthropometric, and metabolic dependent variables

	Log (VLDL-TG FCR)			Log (VLDL-TG ASR)		
	Slope	R^2	P	Slope	R^2	P
1/Age	6.39	0.16	0.10	−6.26	0.06	>0.20
Sex (female)	0.023	<0.01	>0.20	0.136	0.06	>0.20
Log (BMI)	−0.724	0.47	0.002	1.27	0.54	0.0005
1/Waist circumference	64.4	0.28	0.05	−73.2	0.12	>0.20
Fasting glucose	−0.187	0.23	0.05	0.157	0.06	>0.20
Log (fasting insulin)	−0.120	0.18	0.08	0.250	0.29	0.02
Fasting C-peptide	−0.609	0.58	0.0002	0.568	0.19	0.07
Fasting FFA	0.137	0.02	>0.20	−0.498	0.07	>0.20
Log (AIR)	−0.011	<0.01	>0.20	0.199	0.29	0.02
ACR	0.123	0.13	0.14	−0.113	0.04	>0.20
S_I	0.007	0.01	>0.20	−0.046	0.15	0.11
Square root of Cl_{ins}	0.206	0.38	0.007	−0.240	0.19	0.07
Square root of k	1.21	0.16	0.10	−0.969	0.03	>0.20

VLDL-TG FCR were both contributing significantly to total plasma TG (log) ($R^2 = 0.71$, $P < 0.0001$, and $R^2 = 0.27$, $P = 0.03$, respectively), although only VLDL-TG ASR was significantly associated when both variables were included in the model ($R^2 = 0.77$, $P < 0.0001$; $P < 0.0001$ and $P = 0.07$, respectively.

Determinants of VLDL-TG FCR and ASR. Table 5 shows the correlation between VLDL-TG FCR and ASR and the various clinical, anthropometric, and metabolic variables assessed in the present study. BMI (log) (Fig. 2*A*), fasting C-peptide levels (Fig. 2*B*), Cl_{ins} (square root), ACR, and k (square root) (Fig. 2*C*) were all significantly associated with VLDL-TG FCR (log). S_I (Fig. 2*D*), sex, AIR (log), and fasting FFAs did not correlate with VLDL-TG FCR. The best multivariate model to predict VLDL-TG FCR (log) included fasting C-peptide levels, Cl_{ins} (square root), and ACR ($R^2 = 0.79$, $P < 0.0001$; $P = 0.0001$, $P = 0.06$, and $P = 0.07$, respectively). Fasting C-peptide level was the only variable that remained significantly correlated with VLDL-TG FCR when any other variable was included in the model.

BMI (log) (Fig. 3*A*), fasting insulin (log) (Fig. 3*B*), and AIR (log) were significantly positively correlated with VLDL-TG ASR (log). Fasting FFAs (Fig. 3*C*), and k (square root) (Fig. 3*D*) did not correlate with VLDL-TG ASR. The best multivariate model to predict VLDL-TG ASR included BMI (log) and S_I ($R^2 = 0.61$, $P = 0.0008$; $P = 0.0008$ and $P = 0.12$, respectively). Only BMI remained significantly correlated with VLDL-TG ASR when any other variable was included in the model. Correction of VLDL-TG secretion rate for body weight did not affect any of the above associations, and BMI still remained significantly and independently associated with VLDL-TG secretion (not shown).

There were no significant differences in VLDL-apoB, VLDL-TG, VLDL secretion, FCR, S_I, insulin levels, C-peptide levels, FFA levels, or insulin-mediated suppression of plasma FFAs between men and women. Furthermore, forcing sex into the various models did not alter the relationship between BMI, FFAs, S_I, and insulin-mediated suppression of plasma FFA and VLDL levels or secretion.

DISCUSSION

In the present study, BMI alone accounted for 40–65% of the variance of total plasma apoB, plasma TG, VLDL-TG, and VLDL-apoB levels and VLDL-TG secretion rate in our group of participants and was the only factor that remained significantly associated when the effect of any other variable was taken into account. This strong and independent association between BMI and VLDL-TG secretion rate remained even when the latter was normalized for body weight (accounted variance, 37%; data not shown). BMI also accounted for 47% of the variance of VLDL-TG fractional clearance rate, although it was not independent from the effect of fasting C-peptide levels, the main determinant of VLDL-TG FCR in our study. An association between VLDL metabolism and plasma C-peptide levels is in agreement with findings by others (28–30).

Although the magnitude of these associations with BMI is remarkable, these findings are not surprising in view of the well-established association between excess body fat, especially visceral fat, and VLDL levels and secretion rate (31–36). It is generally thought that high FFA flux from visceral adipose tissue, which would not necessarily affect or be reflected by systemic plasma FFA levels, could explain the association between elevated VLDL secretion rate and visceral obesity. In the present study, we used waist circumference as a surrogate marker of visceral adipose tissue mass (37) and found that it was not as well correlated with either VLDL levels or secretion rate as BMI. However, this may simply mean that waist circumference does not have enough accuracy to predict visceral fat mass and that more direct assessment of visceral fat would have been more appropriate to use with our relatively small sample size. Furthermore, it is important to note that we did not measure total body fatty acid flux or the direct influx of FFAs from visceral adipose tissue. The present study measured the sensitivity of FFA output (presumably predominantly from peripheral adipose cells) to insulin suppression. Therefore, we believe that visceral

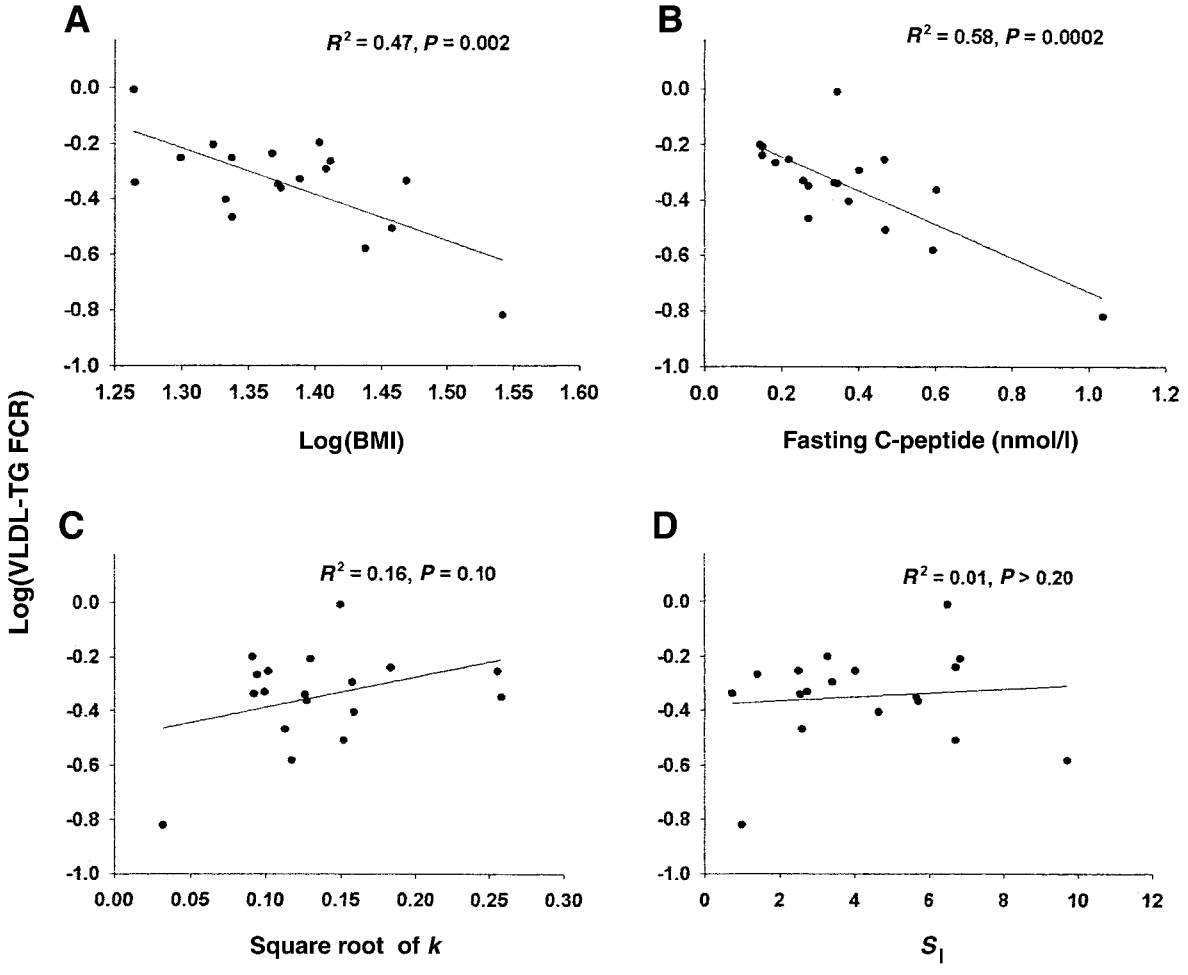

FIG. 2. Correlation between log(VLDL-TG FCR) and log(BMI) (*A*), fasting C-peptide (*B*), square root of insulin-mediated suppression of plasma FFAs (k) (*C*), and S_I (*D*).

fat mass could still explain the association between BMI and VLDL-TG levels and secretion rate in the present study.

The most important and novel finding of the present study is that the sensitivity of insulin-mediated suppression of fasting plasma FFAs was not associated with the rate of postabsorptive VLDL-TG production in healthy individuals. Because we assessed the sensitivity to insulin-mediated suppression of plasma FFAs and not the effect of FFA suppression itself on VLDL secretion, this finding is not in conflict with the generally accepted view that VLDL-TG production is predominantly a substrate-driven process (10). We are in agreement with the observation that an increased flux of FFAs from extrahepatic tissues to the liver plays an important role in the causation of hypertriglyceridemia associated with insulin-resistant states (1,6,10,13,38–43). In addition, our study did not measure the overall channelling of fatty acids toward the liver over a 24-h period. Intracellular lipolysis of adipose tissue triglyceride is much more sensitive to the suppressive effects of insulin than stimulation of peripheral tissue glucose uptake (44), with a half-maximal suppressive dose of insulin on plasma FFA concentration found to be <20 μU/ml (19). Insulin-mediated suppression of plasma FFAs in the postabsorptive state is believed to reflect predominantly inhibition of adipose tissue lipolysis of stored triglycerides, with consequent reduction in the release of FFAs, due to suppression of hormone-sensitive lipase and stimulation of adipose tissue triglyceride reesterification (45). Insulin-mediated suppression of plasma FFAs in the postabsorptive state would not accurately reflect the total 24-h flux of fatty acids to the liver in free-living subjects, where a major contribution occurs from fatty acids derived from ingested fat (46). Fasting plasma FFA levels were not significantly correlated with VLDL levels and secretion in the present study, in accordance with findings by others (28,33,47,48). In contrast, others have observed a significant correlation between postprandial plasma FFA levels and VLDL-TG levels and production rate (47,49). Our findings, therefore, do not preclude an important role for elevated FFA flux to the liver in causing VLDL overproduction in insulin-resistant states, but do suggest that VLDL overproduction is not predicted by impaired insulin-mediated suppression of plasma FFAs in the low insulin-dose range in the postabsorptive state. In other words, impairment of insulin-stimulated plasma FFA esterification in adipose tissue during the postprandial period or impaired suppression of visceral tissue lipolysis may have a greater impact on chronic liver triglyceride secretion than impairment of insulin's antilipolytic effect at the peripheral adipose tissue during fasting. Whether this

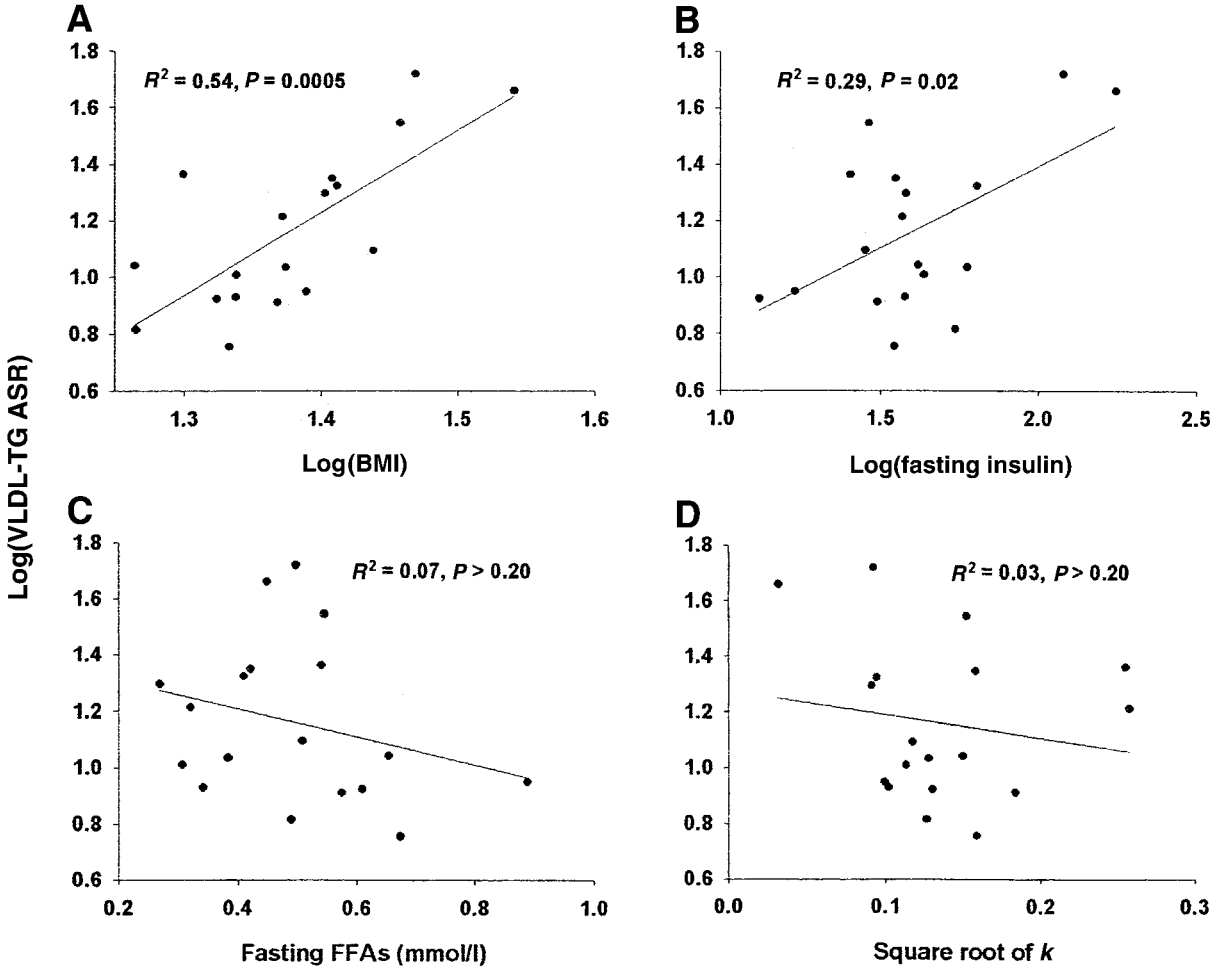

FIG. 3. Correlation between log(VLDL-TG ASR) and log(BMI) (*A*), log(fasting insulin) (*B*), fasting plasma FFAs (*C*), and square root of insulin-mediated suppression of plasma FFAs (*k*) (*D*).

mechanism could perhaps explain some of the association between increased adiposity and VLDL secretion is an unresolved issue. It is important to note that the present study was not designed to explore the mechanism of the association (or lack of association) between insulin-mediated suppression of fasting plasma FFAs and VLDL secretion. More mechanistic in vivo studies are clearly needed to clarify this important issue.

Insulin sensitivity with regard to glucose metabolism (S_I) accounted for only ≤15% of the variance of VLDL levels and kinetics in our study participants, but S_I entered the best model to predict VLDL-TG secretion. However, this association was much less robust than the one with BMI: the significance of the association between S_I or plasma insulin level, but not BMI, and VLDL secretion was lost when we excluded the two subjects with the lowest S_I. Some (28,33,50,51), but not all (35,47), previous kinetic studies have shown a similar degree of association (10–20% of the variance) between fasting insulin levels (surrogate of S_I) or insulin sensitivity per se and VLDL secretion. The findings of the present study further support the concept that insulin resistance to suppression of fasting plasma FFAs and to the stimulation of peripheral tissue glucose uptake may not be the main pathogenic factors causing chronic fasting VLDL oversecretion in the insulin resistance syndrome, but that other associated factors may largely determine the fate of the excess FFA flux to the liver. Previous findings from our group (11,18) and others (52) point to hepatic insulin resistance/hyperinsulinemia as an important determinant of VLDL overproduction. Therefore, while peripheral insulin resistance results in high FFA flux to the liver, liver insulin resistance and/or overinsulinization is likely to be required for the channeling of this excess supply toward VLDL synthesis and secretion and thus to establish clinically significant VLDL overproduction.

In conclusion, neither fasting plasma FFA levels nor the sensitivity of insulin-mediated suppression of plasma FFAs are predictors of fasting VLDL-TG secretion rate in healthy humans. BMI—and to a lesser extent, insulin sensitivity assessed by FSIVGTT with Bergman's minimal model—are the main predictors of fasting VLDL-TG secretion rate in humans. We speculate that obesity may exert its major effects on VLDL production through mechanisms other than its association with extrahepatic insulin resistance, although our studies in no way preclude an important role of elevated postprandial and/or visceral adipose tissue fatty acid flux to the liver in insulin resistance.

ACKNOWLEDGMENTS

This work was funded by a research grant from the Canadian Diabetes Association, and the National Institutes of Health (NIH) Biomedical Mass Spectrometry Resource (NIH RR-00954) and Clinical Nutrition Research Unit (NIH P30 DK56341). A.C. was the recipient of a Medical Research Council/Heart and Stroke Foundation of Canada fellowship and is currently a new investigator of the Canadian Institutes of Health Research. G.F.L. is a Career Investigator of the Heart and Stroke Foundation of Canada and Canada Research Chair in Diabetes (www.chairs.gc.ca).

REFERENCES

1. Lewis GF, Carpentier A, Adeli K, Giacca A: Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23:201–229, 2002
2. Haffner SM, Miettinen H, Gaskill SP, Stern MP: Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. *Diabetes* 44:1386–1391, 1995
3. Grundy SM: Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome. *Am J Cardiol* 81:B18–B25, 1998
4. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna RC, Muggeo M: Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* 47:1643–1649, 1998
5. Howard BV, Mayer-Davis EJ, Goff D, Zaccaro DJ, Laws A, Robbins DC, Saad MF, Selby J, Hamman RF, Krauss RM, Haffner SM: Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Metabolism* 47:1174–1179, 1998
6. Baldeweg SE, Golay A, Natali A, Balkau B, Del Prato S, Coppack SW: Insulin resistance, lipid and fatty acid concentrations in 867 healthy Europeans. European Group for the Study of Insulin Resistance (EGIR). *Eur J Clin Invest* 30:45–52, 2000
7. Lewis GF, Steiner G: Hypertriglyceridemia and its metabolic consequences as a risk factor for atherosclerotic cardiovascular disease in non-insulin-dependent diabetes mellitus. *Diabete Metab Rev* 12:37–56, 1996
8. Taskinen MR: Triglyceride is the major atherogenic lipid in NIDDM (Review). *Diabete Metab Rev* 13:93–98, 1997
9. Lewis GF, Steiner G: Acute effects of insulin in the control of VLDL production in humans: implications for the insulin-resistant state (Review). *Diabetes Care* 19:390–393, 1996
10. Lewis GF: Fatty acid regulation of very low density lipoprotein production. *Curr Opin Lipidol* 8:146–153, 1997
11. Taghibiglou C, Carpentier A, Rudy D, Aiton A, Lewis GF, Adeli K: Mechanisms of hepatic VLDL 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, 2000
12. Coppack SW, Jensen MD, Miles JM: In vivo regulation of lipolysis in humans (Review). *J Lipid Res* 35:177–193, 1994
13. Ginsberg,HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453–458, 2000
14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 22:S5–S19, 1999
15. Lemieux S, Patterson BW, Carpentier A, Lewis GF, Steiner G: A stable isotope method using a [$^2H_5$]glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans. *J Lipid Res* 40:2111–2117, 1999
16. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1985
17. Saad MF, Steil GM, Riad-Gabriel M, Khan A, Sharma A, Boyadjian R, Jinagouda SD, Bergman RN: Method of insulin administration has no effect on insulin sensitivity estimates from the insulin-modified minimal model protocol. *Diabetes* 46:2044–2048, 1997
18. Carpentier A, Patterson BW, Uffelman K, Giacca A, Vranic M, Cattral MS, Lewis GF: Effect of portal vs. systemic insulin delivery in pancreas transplantation on insulin action and VLDL metabolism. *Diabetes* 50:1402–1413, 2001
19. Jensen MD, Caruso M, Heiling V, Miles JM: Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 38:1595–1601, 1989
20. Lookene A, Skottova N, Olivecrona G: Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat). *Eur J Biochem* 222:395–403, 1994
21. Reardon MF, Poapst ME, Uffelman KD, Steiner G: Improved method for quantitation of B apoprotein in plasma lipoproteins by electroimmunoassay. *Clin Chem* 27:892–895, 1981
22. Yang LY, Kuksis A, Myher JJ, Steiner G: Origin of triacylglycerol moiety of plasma very low density lipoproteins in the rat: structural studies. *J Lipid Res* 36:125–136, 1995
23. Patterson BW, Mittendorfer B, Elias N, Satyanarayana R, Klein S: Use of stable isotopically labeled tracers to measure very low density lipoprotein-triglyceride turnover. *J Lipid Res* 43:223–233, 2002
24. Pacini G, Bergman RN: MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 23:113–122, 1986
25. Herman WH, Fajans SS, Smith MJ, Polonsky KS, Bell GI, Halter JB: Diminished insulin and glucagon secretory responses to arginine in nondiabetic subjects with a mutation in the hepatocyte nuclear factor-4alpha/MODY1 gene. *Diabetes* 46:1749–1754, 1997
26. Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ, Bain SC, Hattersley AT, Velho G, Froguel P, Bell GI, Polonsky KS: Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. *Diabetes* 45:1503–1510, 1996
27. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
28. Cummings MH, Watts GF, Pal C, Umpleby M, Hennessy TR, Naoumova R, Sonksen PH: Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in obesity: a stable isotope study. *Clin Sci (Colch)* 88:225–233, 1995
29. Sarlund H, Laakso M, Pyorala K, Penttila I: Association of serum lipids and lipoproteins with plasma C-peptide concentration in non-insulin-dependent diabetic and non-diabetic subjects. *Metabolism* 36:840–845, 1987
30. Kekalainen P, Sarlund H, Laakso M: Long-term association of cardiovascular risk factors with impaired insulin secretion and insulin resistance. *Metabolism* 49:1247–1254, 2000
31. Egusa G, Beltz WF, Grundy SM, Howard BV: Influence of obesity on the metabolism of apolipoprotein B in humans. *J Clin Invest* 76:596–603, 1985
32. Ferland M, Despres JP, Nadeau A, Moorjani S, Tremblay A, Lupien PJ, Theriault G, Bouchard C: Contribution of glucose tolerance and plasma insulin levels to the relationships between body fat distribution and plasma lipoprotein levels in women. *Int J Obes* 15:677–688, 1991
33. Riches FM, Watts GF, Naoumova RP, Kelly JM, Croft KD, Thompson GR: Hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 studied with a stable isotope technique in men with visceral obesity. *Int J Obes* 22:414–423, 1998
34. Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PH: Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. *J Clin Endocrinol Metab* 84:2854–2861, 1999
35. Watts GF, Riches FM, Humphries SE, Talmud PJ, VanBockxmeer FM: Genotypic associations of the hepatic secretion of VLDL apolipoprotein B-100 in obesity. *J Lipid Res* 41:481–488, 2000
36. Zoratti R, Godsland IF, Chaturvedi N, Crook D, Stevenson JC, McKeigue PM: Relation of plasma lipids to insulin resistance, nonesterified fatty acid levels, and body fat in men from three ethnic groups: relevance to variation in risk of diabetes and coronary disease. *Metabolism* 49:245–252, 2000
37. Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP: A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. *Am J Clin Nutr* 64:685–693, 1996
38. Aarsland A, Wolfe RR: Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. *J Lipid Res* 39:1280–1286, 1998
39. Parks EJ, Krauss RM, Christiansen MP, Neese RA, Hellerstein MK: Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 104:1087–1096, 1999
40. Albu JB, Curi M, Shur M, Murphy L, Matthews DE, PiSunyer FX: Systemic resistance to the antilipolytic effect of insulin in black and white women with visceral obesity. *Am J Physiol* 40:E551–E560, 1999
41. Eriksson JW, Smith U, Waagstein F, Wysocki M, Jansson PA: Glucose turnover and adipose tissue lipolysis are insulin-resistant in healthy relatives of type 2 diabetes patients: is cellular insulin resistance a secondary phenomenon? *Diabetes* 48:1572–1578, 1999
42. Abbasi F, McLaughlin T, Lamendola C, Reaven GM: Insulin regulation of

plasma free fatty acid concentrations is abnormal in healthy subjects with muscle insulin resistance. *Metabolism* 49:151–154, 2000
43. Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G: 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, 1995
44. Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630–E639, 1981
45. Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA, Boland O, Coppack SW: Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 266:E308–E317, 1994
46. Frayn KN, Coppack SW, Fielding BA, Humphreys SM: Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. *Adv Enz Reg* 35:163–178, 1995
47. Howard BV, Zech L, Davis M, Bennion LJ, Savage PJ, Nagulesparan M, Bilheimer D, Bennett PH, Grundy SM: Studies of very low density lipoprotein triglyceride metabolism in an obese population with low plasma lipids: lack of influence of body weight or plasma insulin. *J Lipid Res* 21:1032–1041, 1980
48. Howard BV, Abbott WG, Beltz WF, Harper IT, Fields RM, Grundy SM, Taskinen MR: Integrated study of low density lipoprotein metabolism and very low density lipoprotein metabolism in non-insulin-dependent diabetes. *Metabolism* 36:870–877, 1987
49. Boquist S, Hamsten A, Karpe E, Ruotolo G: Insulin and non-esterified fatty acid relations to alimentary lipaemia and plasma concentrations of postprandial triglyceride-rich lipoproteins in healthy middle-aged men. *Diabetologia* 43:185–193, 2000
50. Howard BV, Abbott WG, Egusa G, Taskinen MR: Coordination of very low-density lipoprotein triglyceride and apolipoprotein B metabolism in humans: effects of obesity and non-insulin-dependent diabetes mellitus. *Am Heart J* 113:522–526, 1987
51. Tobey TA, Greenfield M, Kraemer F, Reaven GM: Relationship between insulin resistance, insulin secretion, very low density lipoprotein kinetics, and plasma triglyceride levels in normotriglyceridemic man. *Metabolism* 30:165–171, 1981
52. Koopmans SJ, Kushwaha RS, DeFronzo RA: 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, 1999