Adipocytokines and VLDL Metabolism

Independent Regulatory Effects of Adiponectin, Insulin Resistance, and Fat Compartments on VLDL Apolipoprotein B-100 Kinetics?

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We investigated the relationship of plasma adipocytokine concentrations with VLDL apolipoprotein B (apoB)-100 kinetics in men. Plasma adiponectin, leptin, resistin, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) concentrations were measured using enzyme immunoassays and insulin resistance by homeostasis model assessment (HOMA) score in 41 men with BMI of 22–35 kg/m^2. VLDL apoB kinetics were determined using an intravenous infusion of 1-[^{13}C]leucine, gas chromatography–mass spectrometry, and compartmental modeling. Visceral and subcutaneous adipose tissue mass (ATM) were determined using magnetic resonance imaging, and total ATM was measured by bioelectrical impedance. In univariate regression, plasma adiponectin and leptin concentrations were inversely and directly associated, respectively, with plasma triglyceride; HOMA score; and visceral, subcutaneous, and total ATMs. Conversely, adiponectin and leptin were directly and inversely correlated, respectively, with VLDL apoB catabolism and HDL cholesterol concentration ($P < 0.05$). Resistin, IL-6, and TNF-α were not significantly associated with any of these variables. In multivariate regression, adiponectin was the most significant predictor of plasma VLDL apoB concentration ($P = 0.001$) and, together with total or subcutaneous ATM, was an independent predictor of VLDL apoB catabolism ($P < 0.001$); HOMA score was the most significant predictor of VLDL apoB hepatic secretion ($P < 0.05$). Leptin was not an independent predictor of VLDL apoB kinetics. In conclusion, plasma VLDL apoB kinetics may be differentially controlled by adiponectin and insulin resistance, with adiponectin regulating catabolism and insulin resistance regulating hepatic secretion in men. Total body fat may also independently determine the rate of VLDL catabolism, but leptin, resistin, IL-6, and TNF-α do not have a significant effect in regulating apoB kinetics. *Diabetes* 54: 795–802, 2005

Obesity is an escalating public health problem. It is a major risk factor for atherosclerosis, hypertension, and type 2 diabetes. The risk for cardiovascular disease is highest in obese patients with the metabolic syndrome (1).

The hallmark lipoprotein abnormality in the metabolic syndrome relates to expansion in the plasma pool of triglyceride-rich lipoproteins, in particular in VLDL. The plasma pool of VLDLs is regulated by the hepatic secretion and fractional catabolism of VLDL apolipoprotein B (apoB) and in turn determines the generation of small dense LDL and reduction in plasma HDL concentrations (2). We have previously shown that obesity in men is associated with increased hepatic secretion and delayed catabolism of VLDL apoB and that these kinetic defects are in part related to accumulation of intraperitoneal fat, insulin resistance, and genes that regulate hepatic lipid substrate supply and processing of apoB-100 (3,4).

The precise relationships between dyslipoproteinemia, adiposity, and insulin resistance are complex and undefined (5,6). Recently, there has been intense interest in the notion that a variety of peptides, referred to as adipocytokines, secreted by white adipose tissue can potentially have an impact on glucose and lipid metabolism and contribute to the pathogenesis of the metabolic syndrome (7,8). Rodent data suggest that tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) may regulate insulin sensitivity by stimulating lipolysis and impairing insulin signaling (7). However, plasma levels of TNF-α may not reflect true biological activity, which may principally involve the autocrine regulation of IL-6 secretion from adipocytes. In rodents, IL-6 may also impair insulin signaling in liver and may contribute to hypertriglyceridemia by stimulating hepatic secretion of triglycerides and inhibiting lipoprotein lipase (7). Experimental evidence also suggests that resistin may contribute to insulin resistance (9), but the expression of this adipocytokine in human adipocytes is negligible, and there are no consistent data

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apoB, apolipoprotein B; ATM, adipose tissue mass; FCR, fractional catabolic rate; FFM, fat-free mass; HOMA, homeostasis model assessment; IL-6, interleukin-6; IPATM, intraperitoneal ATM; MRI, magnetic resonance imaging; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator–activated receptor; RPATM, retroperitoneal ATM; SAATM, subcutaneous abdominal ATM; TATM, total ATM; TNF-α, tumor necrosis factor-α.

showing that plasma levels are related to adiposity or insulin sensitivity in humans (7,9,10). By contrast, plasma leptin levels have universally been shown to be positively related to adiposity in humans, but again, a direct effect of leptin on insulin sensitivity in insulin responsive tissue, such as liver, adipocytes, and skeletal muscle, have been less consistent (7); its principal physiological role may be in controlling food intake and thereby regulating body fat stores (11).

In sharp contrast to other adipocytokines, plasma adiponectin levels are decreased in obese and insulin-resistant individuals, including those with type 2 diabetes (12). Experimental and clinical evidence in fact suggests that other adipocytokines may exert their effects on insulin sensitivity by influencing the adipocytic expression and secretion of adiponectin (13,14). Adiponectin may stimulate fatty acid oxidation in skeletal muscle, decreasing intramyocellular accumulation of triglycerides and potentially accelerating the catabolism of triglyceride-rich lipoproteins (15,16); adiponectin may also decrease free fatty acid flux to the liver and hepatic glucose output (17). Hypertriglyceridemia, low HDL cholesterol, and decreased LDL particle size were shown recently in humans to be correlated with low plasma adiponectin levels independent of the degree of intra-abdominal fat and insulin resistance (18–21). The mechanism for the association between plasma adiponectin and dyslipidemia has not been previously investigated. Adiponectin may also have important antiatherogenic and anti-inflammatory properties and has been suggested as a key link between obesity and atherosclerosis (22). The exact mechanisms for the protective effect of adiponectin on atherosclerosis and inflammation are not clear but could involve a direct interaction of plasma adiponectin with the vascular wall that inhibits smooth muscle cell proliferation, lipid accumulation, and endothelial dysfunction (8).

In the present study, we hypothesized that, in men, adiponectin would be the adipocytokine most closely associated with changes in VLDL apoB kinetics and that this association would be independent of body fat compartments and insulin resistance. Our principal aim was to examine the strength and independence of the relationships of VLDL apoB kinetics, measured using stable isotopy, with plasma adiponectin, leptin, resistin, TNF-α, and IL-6 concentrations in relation to body fat compartments and insulin resistance, measured by magnetic resonance imaging (MRI) and homeostasis assessment (HOMA) score, respectively.

RESEARCH DESIGN AND METHODS

We studied 41 white men who had a BMI ranging from 22 to 35 kg/m^2 and no history of familial dyslipidemia or intercurrent illness or were taking drugs that affect lipid metabolism. All were nonsmokers and were consuming ad libitum weight maintenance diets, as previously described (23). Participants were selected from the community. The study was approved by the Royal Perth Hospital Ethics Committee.

Clinical protocols

Clinical tests. Body weight, height, and waist and hip circumference were recorded as previously described (23); BMI and waist-to-hip ratio were calculated. Blood pressure was recorded semiautomatically using a Dinamap recorder (Critilzon, Tampa, FL). Body composition was estimated with the subject at rest in the supine position after emptying the bladder, using a Holtain Body Composition Analyser (Holtain, Dyfed, U.K.) from which total adipose tissue mass (TATM), fat mass, and fat-free mass (FFM) were derived (24); FFM was calculated using the formula of Lukaski et al. (24): FFM = $(0.85 \times H^2/Z) + 3.04$, where H is height (cm) of the subject and Z is impedance. For this measure, participants were also asked to refrain from alcoholic beverages for 24 h; they were then studied in the morning, 15 min after emptying their bladder, and in a temperature-controlled room; the technical error for FFM was <3%.

Dietary and energy expenditure records. Participants completed a 7-day food intake diary that recorded all dietary, alcohol, and energy intake; data were analyzed using DIET4 Nutrient Calculation Software (Xyris Software, Queensland, Australia) based on the Australian Food Composition Database (NUTTAB 95, Australian Government Nutrient Database). Energy expenditure was estimated using a standard 7-day recall questionnaire (25).

MRI. MRI of eight transaxial segments (field of view, 40–48 cm; 10-mm thickness) at intervertebral disc levels from T11 to the S1 was carried out using a 1.0T Picker MR scanner (Picker International, Cleveland, OH) and a T1-weighted fast spin-echo sequence with a high fat-to-water signal ratio (26). Subcutaneous abdominal adipose tissue mass (SAATM), intraperitoneal adipose tissue mass (IPATM), and retroperitoneal adipose tissue mass (RPATM) areas were calculated by summing the relevant adipose tissue pixel units with purpose-designed software, as used earlier (27). Fat anterior to the posterior peritoneum and anterior abdominal wall was defined as IPATM, and fat posterior to the posterior peritoneum was RPATM. Anterior and posterior subcutaneous abdominal compartments were separated by drawing a perpendicular line at the midpoint of the anterior-posterior line passing through midpoints of the vertebral bodies in the MRI images. Anterior subcutaneous ATM was obtained by subtracting posterior SAATM from total SAATM. The rationale for dividing the subcutaneous abdominal fat mass into anterior and posterior compartments was based on the recent studies that posterior (deep) SAATM has a stronger association with peripheral and hepatic insulin sensitivity than both anterior SAATM or IPATM. Further details are described elsewhere (23,28).

Stable isotope test for apoB kinetics. All clinical measurements were carried out after a 14-h fast in a temperature-controlled room in the metabolic ward, and for all stable isotope studies, participants were studied in a semirecumbent position and allowed water only. Isotope was administered via a Teflon cannula placed in a superficial vein of the left antecubital fossa, and 1-[^{13}C]leucine (99.5% enrichment; Tracer Technologies, Somerville, MA) was administered by a primed (1 mg/kg), constant (1 mg · kg^{-1} · h^{-1}) intravenous infusion (10-h duration). Venous blood was collected via a cannula from the contralateral arm without stasis to measure apoB enrichment and concentration. These methods have been previously detailed (3).

Biochemical measurements. VLDL apoB was isolated by preparative ultracentrifugation, precipitated by isopropanol, and quantified by the Lowry method. After hydrolysis and derivatization, isotopic enrichment E(t) of apoB with [^{13}C]leucine was estimated using electron-impact ionization by gas chromatography–mass spectrometry analysis (Hewlett Packard 5890, Wilmington, DE). Tracer:tracee mass units [Z(t)] were used to derive the fractional catabolic rate (FCR) of VLDL apoB using a three-compartment model (3). VLDL apoB secretion rate was estimated by multiplying FCR by pool size, expressed as milligrams per kilogram FFM per day.

Laboratory measurements: adipocytokine assays. Fasting plasma cholesterol, triglyceride, and HDL cholesterol were determined by standard enzymatic methods. LDL cholesterol was calculated using the Friedewald's equation. Plasma nonesterified fatty acids (NEFAs) were measured by an enzymatic colorimetric assay (Boehringer Mannheim, Mannheim, Germany). Plasma insulin was measured by an enzyme-linked immunosorbent assay (Boehringer Mannheim). Plasma glucose concentration was measured by a hexokinase method. Insulin resistance was estimated using the HOMA score (29). Plasma adiponectin, leptin, IL-6, TNF-α, and resistin concentrations were determined using enzyme immunoassay kits (Quantikine; R & D Systems, Minneapolis, MN; Phoenix Pharmaceuticals, Belmont, NY; the interassay coefficients of variation for these methods were <7%).

Statistical analyses. All analyses used SPSS 11.5 (SPSS, Chicago, IL). Skewed data were log transformed where appropriate. Associations of plasma adipocytokines with insulin resistance, plasma lipids and lipoproteins, and adipose tissue compartments were examined by simple, stepwise, and multiple linear regression methods. Because all adipose tissue compartments were highly correlated, they were entered separately into regression models to assess their effects on the relationship of apoB kinetics with adiponectin, leptin, and HOMA score. Because we carried out multiple comparisons, we considered that only $P < 0.01$ was of major importance, while conventionally defining $P < 0.05$ as being statistically significant.

RESULTS

Table 1 shows the clinical and biochemical data relating to the 41 men studied. They were generally middle aged and

TABLE 1
Clinical and biochemical characteristics of the participants studied

Characteristics	Mean ± SD	Range
Age (years)	47.0 ± 8.6	25–61
Systolic blood pressure (mmHg)	127 ± 16	96–164
Diastolic blood pressure (mmHg)	76 ± 10	53–96
Weight (kg)	96.9 ± 12.4	66.6–117
BMI (kg/m^2)	30.4 ± 3.3	22.1–34.9
Waist-to-hip ratio	1.00 ± 0.05	0.87–1.09
FFM (kg)	63.2 ± 7.6	40.1–82.7
IPATM (kg)	3.68 ± 1.50	1.21–8.25
RPATM (kg)	0.50 ± 0.056	0.11–3.73
SAATM (kg)	4.03 ± 1.40	1.40–6.85
Anterior SAATM (kg)	1.41 ± 0.63	0.19–2.91
Posterior SAATM (kg)	2.62 ± 0.87	0.84–4.36
TATM (kg)	33.4 ± 9.9	13.1–56.1
Cholesterol (mmol/l)	5.73 ± 0.93	3.8–8.3
Triglyceride (mmol/l)	2.70 ± 1.93	0.5–8.8
HDL cholesterol (mmol/l)	1.01 ± 0.26	0.6–1.8
LDL cholesterol (mmol/l)	3.59 ± 0.85	1.5–5.8
Non-HDL cholesterol (mmol/l)	4.73 ± 0.95	3.1–7.4
Glucose (mmol/l)	5.41 ± 0.60	4.1–6.9
Insulin (mU/l)	11.68 ± 8.51	2.6–41.8
HOMA score	2.89 ± 2.26	0.55–10.77
Adiponectin (μg/ml)	4.43 ± 2.38	1.39–11.41
Leptin (ng/ml)	1.84 ± 0.37	0.95–2.27
Resistin (ng/ml)	20.41 ± 6.92	5.32–36.63
TNF-α (pg/ml)	1.66 ± 0.68	0.59–3.92
IL-6 (pg/ml)	1.21 ± 0.52	0.40–3.03
VLDL apoB concentration (mg/l)	19.4 ± 16.7	3.40–86.5
VLDL apoB pool size (mg)	87.0 ± 76.2	10.2–384
VLDL apoB secretion (mg · kg FFM^{-1} · day^{-1})	19.3 ± 12.8	3.2–55.0
VLDL apoB catabolism (pools/day)	5.97 ± 5.51	1.01–30.91

normotensive; 28 were obese (BMI ≥30 kg/m^2) and 13 were nonobese. Twenty had the metabolic syndrome by National Cholesterol Education Program Adult Treatment Panel-III criteria (30). IPATM, RPATM, and SAATM comprised 11, 1.5, and 12.1%, respectively, of TATM. Of total SAATM, 35% was in the anterior and 65% was in the posterior compartment. On average, the men were dyslipidemic, with elevated triglycerides and low HDL cholesterol, and insulin resistant. Four participants had impaired fasting glucose (between 6.1 and 6.9 mmol/l). Dietary intake per day (means ± SD) was as follows: energy 9,276 ± 2030 kJ, total fat 36 ± 7%, carbohydrate 38 ± 8%, protein 21 ± 4%, alcohol 6 ± 6%, and cholesterol 385 ± 176 g; daily energy expenditure was 15008 ± 3,151 kJ.

Table 2 shows the univariate associations of plasma adipocytokine concentrations with measures of insulin resistance and adipose tissue compartments. With the exception of NEFAs, glucose, and RPATM, plasma adiponectin concentration was significantly and negatively correlated with plasma insulin, HOMA score, and all adipose tissue compartments, including total body fat. By contrast, plasma leptin concentration was positively associated with glucose, insulin, HOMA score, and all adipose tissue compartments, except for RPATM. Plasma resistin and NEFA concentrations were not significantly correlated with any variables. Plasma TNF-α and IL-6 concentrations were only significantly correlated (positively) with IPATM. Plasma insulin concentration and HOMA score were both positively associated with all adipose tissue compartments, with the exception of RPATM. In stepwise regression, the most significant predictor of adiponectin was the posterior SAATM ($P = 0.001$), and the most significant predictor of leptin was total SAATM ($P < 0.001$).

Table 3 shows the univariate associations of plasma lipid and lipoprotein concentrations and VLDL apoB kinetics with plasma adipocytokines, measures of insulin resistance, and adipose tissue compartments. In these analyses, the most important correlations are those with $P < 0.01$. Plasma adiponectin concentration was significantly and inversely associated with triglycerides, cholesterol, and VLDL apoB concentrations and positively correlated with plasma HDL cholesterol levels and VLDL apoB FCR (both $P < 0.01$). Plasma leptin concentration was significantly and positively associated with triglyceride concentration and VLDL apoB concentration and inversely correlated with HDL cholesterol concentration and VLDL apoB FCR (both $P < 0.05$). Plasma resistin, TNF-α, IL-6, and NEFA concentrations were not significantly correlated with plasma lipid and lipoprotein concentrations or with VLDL apoB kinetics. Plasma insulin concentrations and HOMA score both were significantly positively associated with plasma triglyceride and cholesterol concentrations and with VLDL apoB concentration and secretion rate; these measures of insulin resistance were significantly and inversely correlated with HDL cholesterol and VLDL apoB FCR ($P < 0.05$). The masses of all adipose tissue compart-

TABLE 2
Associations (Pearson correlation coefficients) of plasma adipocytokine concentrations with measures of insulin resistance and adipose tissue compartments

	Adiponectin	Leptin	Resistin	TNF-α	IL-6	NEFA	Glucose	Insulin	HOMA score
NEFAs	0.057	−0.154	0.198	0.152	−0.064	–	0.025	−0.257	−0.295
Glucose	−0.192	0.336†	−0.127	0.157	0.081	0.025	–	–	–
Insulin	−0.552*	0.544†	−0.191	0.028	0.06	−0.257	–	–	–
HOMA score	−0.460*	0.544*	−0.268	0.099	0.062	−0.295	–	–	–
IPATM	−0.389†	0.621*	0.016	0.332†	0.419†	−0.278	0.447*	0.594*	0.617*
RPATM	0.092	0.188	0.024	0.024	0.030	−0.021	0.161	0.121	0.038
Total SAATM	−0.500*	0.731*	0.218	0.030	0.077	−0.187	0.265	0.481*	0.486*
Anterior	−0.422*	0.617*	0.144	−0.037	0.012	−0.191	0.217	0.371†	0.377†
Posterior	−0.502†	0.734*	0.248	0.076	0.115	−0.164	0.271	0.509*	0.513*
TATM	−0.421*	0.707*	0.176	0.106	0.252	−0.294	0.166	0.510*	0.498*

*$P < 0.01$; †$P < 0.05$.

TABLE 3
Associations (Pearson correlation coefficients) of plasma lipid and lipoprotein concentrations and VLDL apoB kinetics with plasma adipocytokine concentrations, measures of insulin resistance, and adipose tissue compartments

	Triglyceride	Cholesterol	HDL cholesterol	LDL cholesterol	VLDL apoB concentration	VLDL apoB secretion	VLDL apoB catabolism
Adiponectin	−0.632*	−0.453*	0.474*	−0.172	−0.622*	−0.153	0.536*
Leptin	0.548*	0.224	−0.414*	0.050	0.463†	0.137	−0.483†
Resistin	0.034	0.007	0.120	−0.009	−0.010	−0.177	−0.088
TNF-α	0.198	0.197	−0.105	0.119	0.187	0.132	−0.130
IL-6	0.169	0.132	−0.060	0.149	0.167	0.066	−0.248
NEFAs	−0.197	−0.078	0.163	−0.063	−0.167	−0.151	−0.259
Glucose	0.401*	0.016	−0.370†	−0.135	0.367†	0.351†	−0.045
Insulin	0.660*	0.423*	−0.439*	0.119	0.572*	0.365†	−0.389†
HOMA score	0.671*	0.395*	−0.459*	0.092	0.583*	0.388†	−0.368†
IPATM	0.565*	0.266	−0.366†	0.022	0.553*	0.354†	−0.403*
RPATM	0.438*	0.206	−0.285	0.025	0.310†	0.169	−0.246
Total SAATM	0.591*	0.317†	−0.433*	0.132	0.517*	0.027	−0.522*
Anterior	0.546*	0.255	−0.340*	0.018	0.452*	0.025	−0.452*
Posterior	0.560*	0.328†	−0.453*	0.195	0.508*	0.025	−0.516*
TATM	0.468*	0.301	−0.309†	0.173	0.490*	0.015	−0.609*

*$P < 0.01$; †$P < 0.05$.

ments were significantly and positively associated with plasma triglyceride concentration and VLDL apoB concentration ($P < 0.05$) and, with the exception of RPATM, were also negatively correlated with plasma HDL cholesterol and VLDL apoB FCR ($P < 0.05$). Only IPATM was significantly and positively associated with VLDL apoB secretion ($P < 0.05$). Dietary intake and energy expenditure were not significantly correlated with plasma lipids, adipocytokines, and measures of insulin resistance or apoB kinetics (data not shown). As shown in Table 4, plasma adiponectin concentration was a significant, independent predictor of the VLDL apoB concentration in regression models including HOMA score, age, and IPATM (model 1; adjusted $R^2 = 48\%$, $P < 0.001$), total SAATM (model 2; adjusted $R^2 = 45\%$, $P < 0.001$), and TATM (model 3; adjusted $R^2 = 45\%$, $P < 0.001$). Adiponectin was also an independent predictor of VLDL apoB concentration in regression models including anterior and posterior SAATM. In stepwise regression, plasma adiponectin concentration was also the best predictor of VLDL apoB (β-coefficient = −0.546, $P < 0.001$), triglyceride (β-coefficient = −0.620, $P < 0.001$), and HDL cholesterol concentrations (β-coefficient = 0.508, $P < 0.001$). Exclusion of four participants with plasma triglyceride concentration >4.5 mmol/l from the analyses did not alter the significant association of plasma adiponectin with other variables shown in Tables 2 and 3.

As shown in Table 4, insulin resistance, as measured by the HOMA score, was an independent predictor of VLDL apoB secretion rate in a multiple regression model that included plasma adiponectin, age, and IPATM (model 1; adjusted $R^2 = 14\%$, $P = 0.049$), total SAATM (model 2; adjusted $R^2 = 11\%$, $P = 0.08$), and TATM (model 3; adjusted $R^2 = 6\%$, $P = 0.185$). Inclusion of total, posterior, or anterior SAATM in the models did not alter the significance of the association between HOMA score and VLDL apoB secretion rate. In stepwise regression, the HOMA score was the most significant predictor of the VLDL apoB secretion rate (β-coefficient = 0.388; $P < 0.05$).

Table 4 shows that plasma adiponectin concentration was a significant independent predictor of the VLDL apoB FCR in a regression model that included HOMA score, age, and IPATM (model 1; adjusted $R^2 = 23\%$, $P = 0.008$), total SAATM (model 2; adjusted $R^2 = 33\%$, $P = 0.001$), and TATM (model 3; adjusted $R^2 = 41\%$, $P < 0.001$). In these models, total SAATM and TATM were also independent predictors of VLDL apoB FCR. Inclusion of posterior SAATM or anterior SAATM instead of total SAATM in model 2 did not alter the significance of the association between plasma adiponectin concentrations and VLDL apoB FCR. In stepwise regression that included these compartments, plasma adiponectin concentration was the most significant predictor of the VLDL apoB FCR (β-coefficient = 0.641; $P < 0.001$). Moreover, inclusion of plasma leptin in any of the regression models (Table 4) did not attenuate the effect of adiponectin on VLDL apoB FCR.

In relation to the models given in Table 4, Fig. 1 demonstrates the associations of VLDL apoB kinetic parameters with plasma adiponectin concentrations (*A–C*), HOMA score (*D–F*), and TATM (*E–I*). The scatterplots for total SAATM were similar to those for TATM.

By contrast to adiponectin, plasma leptin was not an independent predictor of VLDL apoB kinetics in regression models that included HOMA score, age, and adipose tissue compartments (Table 5). In these models, HOMA score was the only significant predictor of VLDL apoB concentration and secretion rate.

DISCUSSION

This is the first study of the relationships between plasma adipocytokines, insulin resistance, body fat compartments, and VLDL apoB kinetics in humans. Our principal result was that low plasma adiponectin levels were highly predictive of elevated plasma VLDL apoB concentration and that this was chiefly related to impaired catabolism of VLDL apoB. This potential effect of adiponectin was independent of both insulin resistance and size of adipose tissue compartments. Hepatic production of VLDL apoB, however, was most significantly related to insulin resistance and IPATM. Either other adipocytokines were not significantly associated with VLDL apoB kinetics, or, in the

TABLE 4
Multiple regression analyses of the relationship between plasma adiponectin with VLDL apoB concentration, secretion, and catabolism, adjusting for HOMA, age, and ATMs

Predictor variable	Regression coefficient	SE	*P* value
A. VLDL apoB concentration			
Model 1*			
Adiponectin (μg/ml)	**−0.434**	**0.221**	**0.004**
HOMA score	0.164	0.177	0.322
Age (years)	0.052	0.005	0.665
IPATM (kg)	0.289	0.034	0.055
Model 2			
Adiponectin (μg/ml)	**−0.384**	**0.238**	**0.015**
HOMA score	0.267	0.163	0.083
Age (years)	0.089	0.05	0.468
Total SAATM (kg)	0.200	0.036	0.173
Model 3			
Adiponectin (μg/ml)	**−0.414**	**0.229**	**0.007**
HOMA score	0.247	0.167	0.116
Age (years)	0.052	0.005	0.665
TATM (kg)	0.204	0.005	0.149
B. VLDL apoB secretion†			
Model 1			
Adiponectin (μg/ml)	0.166	0.911	0.332
HOMA score	**0.371**	**1.02**	**0.046**
Age (years)	0.040	0.225	0.790
IPATM (kg)	0.226	1.50	0.206
Model 2			
Adiponectin (μg/ml)	0.075	0.984	0.682
HOMA score	**0.480**	**0.975**	**0.008**
Age (years)	0.049	0.230	0.753
Total SAATM (kg)	−0.099	1.615	0.578
Model 3			
Adiponectin (μg/ml)	0.022	0.233	0.909
HOMA score	**0.461**	**0.170**	**0.028**
Age (years)	−0.022	0.005	0.889
TATM (kg)	−0.280	0.005	0.130
C. VLDL apoB catabolism‡			
Model 1			
Adiponectin (μg/ml)	**0.479**	**0.246**	**0.007**
HOMA score	0.029	0.197	0.886
Age (years)	−0.063	0.005	0.661
IPATM (kg)	−0.189	0.038	0.293
Model 2			
Adiponectin (μg/ml)	**0.386**	**4.13**	**0.025**
HOMA score	0.033	2.83	0.843
Age (years)	−0.155	0.087	0.256
TSAATM (kg)	**−0.357**	**0.628**	**0.032**
Model 3			
Adiponectin (μg/ml)	**0.402**	**0.219**	**0.011**
HOMA score	0.127	0.159	0.429
Age (years)	−0.121	0.005	0.342
TATM (kg)	**−0.502**	**0.005**	**0.001**

*Adjusted R^2: model 1 48%, $P = 0.001$; model 2 45%, $P = 0.001$; model 3 45%, $P = 0.001$; †adjusted R^2: model 1 14.2%, $P = 0.049$; model 2 11%, $P = 0.08$; model 3 6%, $P = 0.185$; ‡adjusted R^2: model 1 23%, $P = 0.008$; model 2 33%, $P = 0.001$; model 3 41%, $P < 0.001$. Boldface indicates significant associations.

case of leptin, significant associations were not independent of body fat compartments and insulin resistance.

Our findings provide a kinetic basis for previously reported associations of low plasma adiponectin concentrations with elevated triglycerides, low HDL cholesterol, and small LDL particle size. Cross-sectional studies have shown that these relationships are independent of insulin resistance, measured by either an insulin clamp or HOMA score (18,19). In agreement with other data, we suggest that the potential impact of adiponectin on dyslipidemia is independent of ATM, noting that previous studies have not carried out as comprehensive an investigation of body fat compartments. Because a previous investigation demonstrated that plasma adiponectin levels were dependent on age and sex, we restricted our investigation to middle-aged men, a group in which the metabolic syndrome is especially common (19). Dyslipidemia in insulin resistance is fundamentally related to expansion in the plasma pool of triglyceride-rich VLDLs; this primary abnormality in turn increases the catabolism of HDL and the production of small dense LDL as a consequence of triglyceride enrichment of these lipoproteins via the action of cholesteryl ester transfer protein and the subsequent lipolytic effect of hepatic lipase (2,5). By contrast to others, we found that the reciprocal relationship of adiponectin with dyslipidemia and, in particular, VLDL apoB concentration was independent of IPATM. Cnop et al. (19), however, did not measure VLDL apoB kinetics, and we explain their findings as being related to a dominant effect of low plasma adiponectin levels in impairing the catabolism of VLDL apoB. We confirm our previous observations that insulin resistance is a significant positive predictor of hepatic secretion of VLDL apoB (3,27). Although this association was independent of IPATM, it was only marginally statistically significant in regression analyses. Nevertheless, discrepancies with previous studies may be due to differences in sample sizes and population characteristics. That we found no significant correlations between plasma NEFAs and VLDL apoB secretion rate suggests that circulating NEFA levels do not reflect the flux of NEFAs supplied by IPATM via the portal vein to the liver. Our data also showed that together with plasma adiponectin levels, TATM was an independent predictor of VLDL apoB catabolism; this supports the notion that catabolism of VLDL apoB particles is dependent on both the activity of lipoprotein lipase and skeletal muscle blood flow, both of which are reduced with increasing total body adiposity (31). Our data suggest that plasma adiponectin and TATM could independently have an impact on VLDL apoB catabolism via different mechanisms. Although total SAATM was an independent predictor of FCR, it was not a predictor of VLDL apoB concentration. It is likely that the effect of total SAATM on apoB concentration might have been overridden by the effects of adiponectin and insulin resistance on VLDL apoB secretion and catabolism, respectively (see Table 4).

The mechanism for the effect of adiponectin on VLDL apoB catabolism may involve principally its effects on skeletal muscle lipid metabolism and probably an indirect effect on lipoprotein lipase activity in both skeletal muscle and adipocytes (15). The catabolism of VLDL apoB is partly controlled by the insulin sensitivity, triglyceride content, and local fatty acid concentrations in skeletal muscle. Adiponectin may decrease the accumulation of triglycerides and the concentrations of fatty acids in skeletal muscle by enhancing fatty acid oxidation through activation of acetyl CoA oxidase, carnitine palmitoyltransferase-1, and AMP kinase (15). Adiponectin may also indirectly stimulate lipoprotein lipase (32), the lipolytic enzyme that

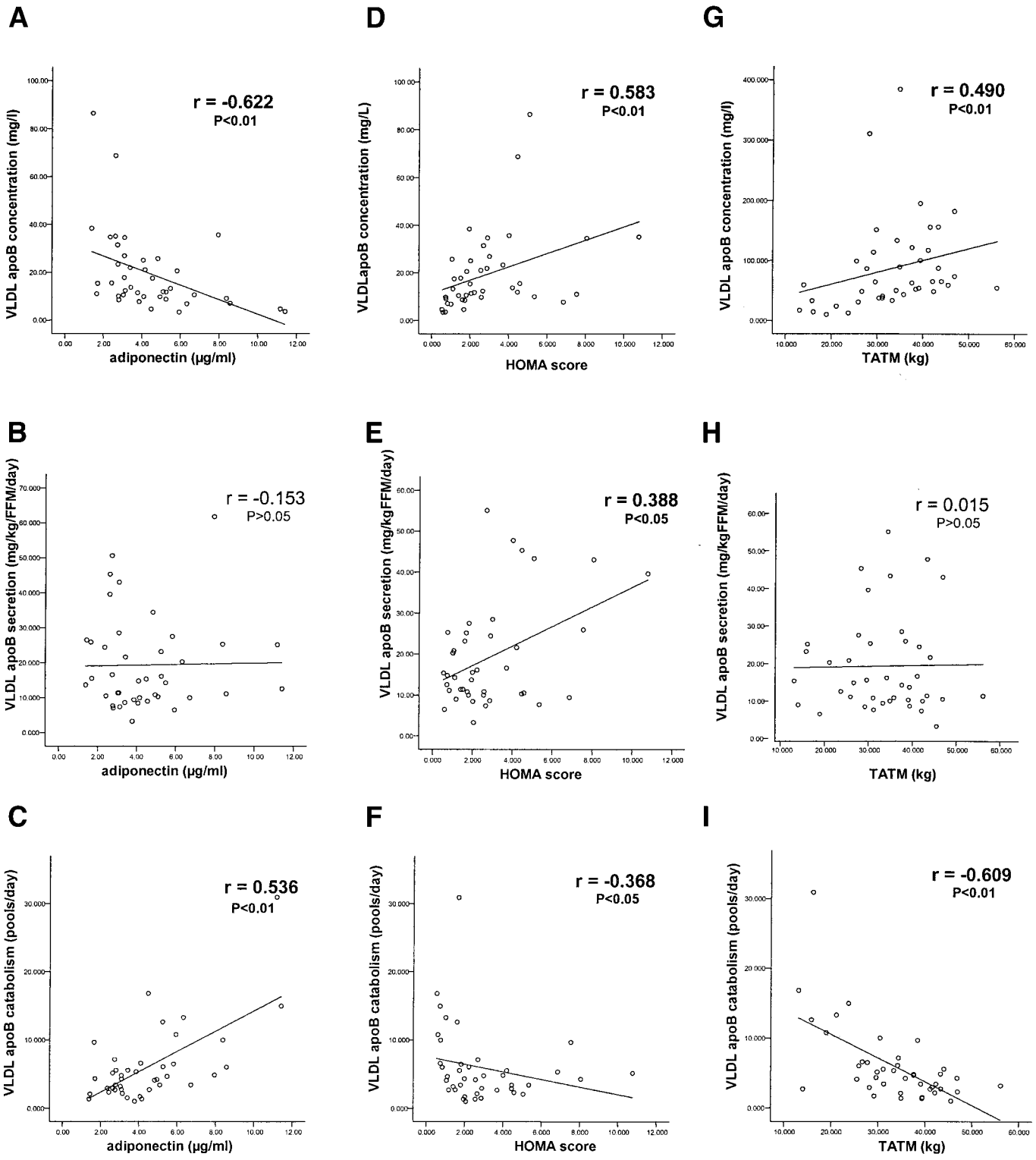

FIG. 1. Associations of VLDL apoB kinetic parameters with plasma adiponectin concentration (*A–C*), HOMA score (*D–F*), and TATM (*G–I*). In stepwise regression including for FCR and secretion rate, FCR is the most significant predictor of VLDL apoB concentration (adjusted R^2 = 41%, P < 0.001).

catabolizes VLDL by increasing the expression of peroxisome proliferator–activated receptor (PPAR)-α in the liver and adipocytes (33). However, our suggestion of a predominant skeletal muscle effect of depressed plasma adiponectin levels is consistent with decreased activation of the adiponectin receptor R1 related to reduced circulating concentration of the C-terminal globular domain of the peptide (34). Because resistin, IL-6, and TNF-α were not associated with insulin resistance and total body fat in the present study, it was not surprising that we found no significant association of these peptides with VLDL apoB kinetics. Resistin is negligibly expressed in mature human

TABLE 5
Multiple regression analyses of the relationships of VLDL apoB concentration (A), secretion (B), and catabolism (C), with plasma leptin HOMA score, age, and adipose tissue compartments

Predictor variable	Regression coefficient	SE	*P*e
A. VLDL apoB concentration*			
Leptin (ng/ml)	0.097	0.598	0.576
HOMA	**0.366**	**0.185**	**0.038**
Age (years)	0.097	0.598	0.576
IPATM (kg)	0.270	0.042	0.143
B. VLDL apoB secretion†			
Leptin (ng/ml)	−0.221	24.19	0.254
HOMA score	**0.352**	**0.981**	**0.049**
Age (years)	0.083	0.222	0.580
Total SAATM (kg)	0.303	1.684	0.133
C. VLDL apoB catabolism‡			
Leptin (ng/ml)	−0.133	12.39	0.563
HOMA score	−0.096	2.881	0.573
Age (years)	−0.082	0.096	0.563
TATM (kg)	0.387	0.861	0.085

*Adjusted R^2 = 34%, P = 0.001; †adjusted R^2 = 15%, P = 0.041; ‡adjusted R^2 = 23%, P = 0.009.

adipocytes, and its roles in insulin resistance are unclear (10). Both TNF-α and IL-6 can have autocrine effects that decrease adiponectin secretion from adipocytes, but this potential mechanism may not be reflected by the corresponding circulating plasma levels of these peptides. We confirm previous reports that plasma leptin levels are directly related to body fat stores and to insulin resistance (11,35). However, we show that the association of leptin with VLDL apoB kinetics is not independent of these two variables. As suggested elsewhere, elevated expression and concentrations of plasma leptin in obesity may reflect only body fat stores and hence may not per se have a direct impact on insulin resistance or apoB kinetics (11).

Although adiponectin seems to regulate plasma VLDL apoB concentrations at a catabolic level, we confirm our previous observations that the hepatic output of VLDL apoB is significantly and independently related to insulin resistance, as reflected by hyperinsulinemia and elevated HOMA score (27). The associated mechanisms involved increase in free fatty acid flux to the liver, resistance to a direct inhibitory effect of insulin on apoB secretion, decreased posttranslational degradation of apoB, increased expression of microsomal triglyceride transfer protein and SREBP-1c (sterol regulatory element–binding protein-1c), and decreased expression of PPAR-γ (5,36). In addition to these molecular mechanisms, insulin resistance may operate in concert with low plasma adiponectin levels to impair the catabolism of triglyceride-rich lipoproteins by lipoprotein lipase (37). This metabolic defect may also have an impact on the clearance of exogenously derived lipoproteins, such as chylomicrons and remnants, by competing for common removal pathways.

Our study is limited by its cross-sectional design. Definitive evidence of the role of adiponectin in regulating apoB metabolism will require further investigation using adiponectin knockout animals and recombinant adiponectin replacement therapy. Although we reported significant associations in the present study, a large proportion of the variation in apoB kinetics remained unexplained. We attempted to account for variation in nutrient intake and exercise, but this was based on historical details. Our estimate of insulin resistance was the HOMA score; a more accurate measurement of skeletal muscle insulin resistance would require use of the hyperinsulinemic-euglycemic clamp. We did not measure postheparin lipoprotein lipase activity that otherwise may provide a more precise mechanism of action of plasma adiponectin on VLDL apoB catabolism. The expression of circulating levels of adiponectin has a strong genetic component (38–40), so further studies should examine the effect of adiponectin genotypes on apoB kinetics. The role of genetic variations in adiponectin receptor subtypes and relationships with other genotypes that regulate lipid substrate supply are clearly important related questions. An unresolved issue is whether the association of plasma adiponectin concentrations with VLDL apoB kinetics in the present study reflects the effect of the full-length peptide or C-terminal globular form, because our assay does not distinguish between these two forms of circulation adiponectin levels. Adiponectin also exists in plasma as high and low molecular isoforms. The former is more closely associated with insulin sensitivity (39), but whether this also applies to VLDL apoB kinetics remains to be investigated. Our study was also restricted to VLDL apoB kinetics, and further investigations should explore the association of adiponectin levels or adiponectin replacement therapy with intermediate-density lipoprotein, LDL, and HDL kinetics. The strength of the correlations in our study between adiponectin and elevated triglyceride on the one hand and low HDL cholesterol on the other hand suggests that low adiponectin may be associated with increased generation of small dense LDL particles as well as higher catabolism of HDL apoAI (41,42).

Adiponectin has recently been demonstrated to have several antiatherogenic functions, involving antiadhesive, antiproliferative, and antioxidant properties (8,40). Our study suggests that beyond these effects, adiponectin also significantly and independently regulates the plasma concentration of VLDL apoB by controlling its rate of catabolism with important mechanistic implications for the impact of increasing adiposity on the atherogenic lipoprotein phenotype typical of the metabolic syndrome. Clinical studies relating changes in plasma adiponectin levels to apoB kinetics in response to weight loss and PPAR agonists in male and female subjects are warranted. The impact of recombinant adiponectin therapy on lipoprotein kinetics in individuals with the metabolic syndrome is also required to confirm definitively the hypotheses of our present study.

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