Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes

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Abstract Patients with type 2 diabetes have high levels of triglyceride-rich lipoproteins (TRLs), including apolipoprotein B-48 (apoB-48)-containing TRLs of intestinal origin, but the mechanism leading to overaccumulation of these lipoproteins remains to be fully elucidated. Therefore, the objective of this study was to examine the in vivo kinetics of TRL apoB-48 and VLDL, intermediate density lipoprotein (IDL), and LDL apoB-100 in type 2 diabetic subjects (n = 11) and nondiabetic controls (n = 13) using a primed-constant infusion of L-[5,5,5-D_3]leucine for 12 h in the fed state. Diabetic subjects had significantly higher fasting glycemia, higher fasting insulinemia, higher plasma triglyceride, and lower HDL-cholesterol levels than controls. Compared with controls, diabetic subjects had increased TRL apoB-48, VLDL apoB-100, and IDL apoB-100 pool sizes as a result of increased production rates (PRs) and reduced fractional catabolic rates of these lipoprotein subfractions. Furthermore, multiple linear regression analyses revealed that the diabetic/control status was an independent predictor of TRL apoB-48 PR and represented nearly 35% of its variance. These results suggest that the overaccumulation of TRLs seen in patients with type 2 diabetes is attributable to increased PRs of both intestinally derived apoB-48-containing lipoproteins and TRL apoB-100 of hepatic origin and to decreased catabolism of these subfractions.— Hogue, J-C., B. Lamarche, A. J. Tremblay, J. Bergeron, C. Gagné, and P. Couture. **Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. *J. Lipid Res.* 2007.** 48: **1336–1342.**

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Type 2 diabetes is a complex disease known to increase triglyceride (TG) levels, decrease HDL-cholesterol levels, and promote the formation of small, dense LDL particles. Recent studies indicate that type 2 diabetes is associated with increased levels of triglyceride-rich lipoproteins (TRLs), including apolipoprotein B-48 (apoB-48)-containing TRLs of intestinal origin, in both the fasted and postprandial states (1, 2). This is of interest because substantial evidence exists indicating that high levels of intestine-derived lipoproteins are associated with increased cardiovascular disease risk (3). Chylomicrons are too large to be able to enter the subendothelial space, but once hydrolyzed by the lipoprotein lipase, chylomicron remnants of <700 Å are small enough to enter into the intima and to participate in atherosclerotic lesion development (4). Chylomicron remnants have been shown to impair normal endothelial function (5), to be chemically modified, and to accumulate in the subendothelial space in the same way as apoB-100-containing lipoproteins do (6, 7). However, the mechanisms underlying the overaccumulation of apoB-48-containing TRLs in type 2 diabetes have not been fully characterized. Studies have suggested a mixed contribution of VLDL overproduction leading to competition for the removal of apoB-48-containing TRLs, impaired lipoprotein lipase activity, modified composition of TRLs, and reduced recognition by hepatic receptors (8–10). Furthermore, a recent study (11) suggested that hyperinsulinemia was associated with an increased production rate (PR) of apoB-48-containing lipoproteins of intestinal origin in insulin-resistant humans. That study, however, did not specifically examine the impact of insulin resistance on TRL apoB-48 metabolism in subjects with type 2 diabetes. Therefore, the objectives of this study were to determine whether intestinal apoB-48-containing lipoprotein secretion and catabolism are impaired in patients with type 2 diabetes and severe hypertriglyceridemia and to examine correlations between apoB-48 and apoB-100 kinetics.

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METHODS

Subjects

Eleven men with type 2 diabetes and severe hypertriglyceridemia and 13 normolipidemic nondiabetic controls were included in this study. Diabetic subjects had to suffer from type 2 diabetes as defined by the American Diabetes Association (12) and to have a clinical requirement for an oral hypoglycemic agent. All diabetic patients were treated with either metformin or a combination of metformin and a sulfonylurea. Three subjects received a thiazolidinedione. For all subjects, exclusion criteria were as follows: history of cardiovascular disease; microalbuminuria; a genetic condition affecting lipid metabolism (e.g., familial hypercholesterolemia, type III hyperlipidemia, LPL deficiency, etc.); body mass index (BMI) of <18.0 or >35.0 kg/m^2; uncontrolled hypothyroidism; nephrotic syndrome; anorexia nervosa; history of alcohol or drugs abuse; persistent increased alanine aminotransferase, aspartate aminotransferase, or creatine phosphokinase; uncontrolled endocrine or metabolic disease; poor mental condition; or a positive test for human immunodeficiency virus. All diabetic subjects had to receive stable doses of oral hypoglycemic agents for at least 3 months before the study to achieve glycosylated hemoglobin (HbA_1C) values of $<9\%$. Lipid-lowering medications were withdrawn for at least 6 weeks before the kinetic study. Upon their entry into the study, subjects met with a dietician and were instructed to maintain their usual nutritional habits throughout the entire intervention. A standardized food frequency questionnaire was also administered to participants to estimate their diet composition, and no significant difference was observed between the two groups. Fasting plasma insulin and glucose levels were measured to compute the insulin resistance index based on the homeostasis model assessment model (13). The research protocol was approved by the Laval University Medical Center review committee, and written informed consent was obtained from each subject.

Experimental protocol for in vivo stable isotope kinetics

To determine the kinetics of TRL apoB-48 and VLDL, intermediate density lipoprotein (IDL), and LDL apoB-100, participants underwent a primed-constant infusion of L-[5,5,5-D_3]leucine while they were in a constantly fed state. Starting at 7 AM, the subjects received 30 identical small cookies every half-hour for 15 h, each equivalent to 1/30th of their estimated daily food intake based on the Harris-Benedict equation (14), with 15% of calories as protein, 45% as carbohydrate, and 40% as fat (7% saturated, 26% monounsaturated, and 7% polyunsaturated) and 85 mg of cholesterol/1,000 kcal. At 10 AM, with two intravenous lines in place, one for the infusate and one for blood sampling, L-[5,5,5-D_3]leucine (10 μmol/kg body weight) was injected as a bolus and then by continuous infusion (10 μmol/kg body weight/h) over a 12 h period. Blood samples (20 ml) were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h.

Characterization of fasting plasma lipids and lipoproteins

Before the kinetic studies, 12 h fasting venous blood samples were obtained from an antecubital vein into Vacutainer tubes containing EDTA (0.1% final concentration). Samples were then immediately centrifuged at 4°C for 10 min at 3,000 rpm to obtain plasma and were stored at 4°C until processed. Plasma VLDL ($d < 1.006$ g/ml) were isolated by preparative ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$. The cholesterol and TG contents of the infranatant fraction were measured before and after the precipitation step. Cholesterol and TG levels were determined using an Olympus AU400[e] analyzer (Melville, NY) using reagents and calibrators provided by the manufacturer. ApoB concentrations were measured by nephelometry (Dade Behring, Mississauga, Ontario, Canada) in plasma and in TRL and LDL fractions using reagents and calibrators provided by the manufacturer.

Quantification and isolation of apoB-48 and apoB-100

Venous blood samples were obtained from an antecubital vein into Vacutainer tubes containing EDTA (0.1% final concentration) at various time intervals during the kinetic study. ApoB concentrations were determined in the TRL fraction by noncompetitive ELISA using immunopurified polyclocal antibodies (Alerchek, Inc., Portland, ME) to calculate the pool size (PS). The coefficient of variation for this apoB assay was between 6% and 10%, depending on the region of the standard curve. ApoB-100 and apoB-48 were separated by SDS 3–10% one-dimensional polyacrylamide gradient gel electrophoresis (1D-PAGGE), according to standardized procedures (15), using 50 μl of each sample. ApoB-48 was assessed in the TRL (VLDL) fraction only. Based on the assumption that apoB-48 and apoB-100 both have the same chromogenicity, the relative proportion of each apolipoprotein was assessed by performing a densitometric scan of the gels. We scanned the TRL gels from three different time points to calculate the apoB-48/apoB-100 ratios and to estimate the average concentrations of apoB-100 and apoB-48 in the TRL fraction using the total TRL apoB concentration.

Isotopic enrichment determinations

ApoB-48 and apoB-100 bands were excised from the gels and hydrolyzed in 6.0 N HCl at 110°C for 24 h (16). After heating, the tubes were centrifuged and decanted to remove most of the polyacrylamide. The amino acids were then converted into trifluoromethyloxalizone derivatives using the single-step trifluoroacetic acid/trifluoroacetic anhydride procedure developed by Dwyer et al. (17). The derivatives were analyzed on a Hewlett-Packard 6890/5973 gas chromatograph/mass spectrometer. Isotopic enrichment (%) and tracer-to-tracee ratio (%) were calculated from the observed ion current ratios. The isotopic enrichment of leucine in the apolipoprotein was expressed as tracer-to-tracee ratio (%) using standardized formulas (18).

Kinetic analysis

Kinetics of apoB-48 and apoB-100 were derived by a multicompartmental model as described previously (19). We assumed a constant enrichment of the precursor pool and used either the TRL apoB-48 or the VLDL apoB-100 plateau tracer-to-tracee ratio data as the forcing function to drive the appearance of tracer into apoB-48 and apoB-100, respectively (16). Under steady-state conditions, the fractional catabolic rate (FCR) is equivalent to the fractional synthetic rate. ApoB PRs were determined by the formula PR (mg/kg/day) = [FCR (pools/day) × apoB concentration (mg/l) × plasma volume (liters)]/body weight (kg) (20). Plasma volume was estimated as 4.5% of body weight. The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the observed tracer data.

Statistical analysis

Data from the two groups were compared using Chi-square tests for categorical measures and ANOVA tests for continuous variables. Plasma TG levels were log-transformed to normalize their distribution. Pearson correlation coefficients were determined to assess the significance of associations between parameters. Stepwise multiple linear regression analysis was used to

TABLE 1. Demographic, anthropometric, and biochemical characteristics of subjects according to status

Characteristic	Controls	Diabetics	*P*
Number	13	11	–
Age (years)	36.2 ± 11.3	54.1 ± 7.5	0.0002
BMI (kg/m^2)	26.6 ± 3.0	29.3 ± 3.7	0.06
Fasting glycemia (mmol/l)	4.8 ± 0.4	8.7 ± 2.0	<0.0001
Insulin (pmol/l)	57 ± 14	87 ± 45	0.03
Homeostasis model assessment-IR	2.0 ± 0.6	5.6 ± 2.9	0.0002
Glycosylated hemoglobin (%)	–	7.4 ± 1.0	–

BMI, body mass index; IR, insulin resistant. All subjects were men.

interpret the relationship of these associations. All analyses were performed using JMP Statistical Software (version 6.0; SAS Institute, Cary, NC).

RESULTS

Demographic, anthropometric, and biochemical characteristics of subjects

Table 1 shows the demographic, anthropometric, and biochemical characteristics of subjects according to their diabetic/control status. Diabetic subjects were significantly older than controls (54.1 ± 7.4 vs. 36.2 ± 11.3 years; $P = 0.0002$). There was no significant difference in BMI between the two groups. Diabetic subjects had higher fasting glucose levels (8.7 ± 2.0 vs. 4.8 ± 0.4 mmol/l; $P < 0.0001$), higher fasting insulin levels (87 ± 45 vs. 57 ± 14 pmol/l; $P = 0.03$), and higher homeostasis model assessment-insulin resistant (5.6 ± 2.9 vs. 2.0 ± 0.6; $P = 0.0002$). The mean HbA_1C value in diabetics was 7.4 ± 1.0%.

Table 2 shows the fasting lipid/lipoprotein profiles of subjects according to their status. Diabetic subjects had significantly higher plasma TG (4.60 ± 1.73 vs. 1.23 ± 0.67 mmol/l; $P < 0.0001$) (range, 2.3–8.1 mmol/l), higher TRL cholesterol (2.12 ± 0.97 vs. 0.49 ± 0.32 mmol/l; $P < 0.0001$), higher TRL TG (4.17 ± 1.52 vs. 0.79 ± 0.57 mmol/l; $P < 0.0001$), higher TRL apoB (0.27 ± 0.07 vs. 0.18 ± 0.07 g/l; $P = 0.005$), and lower HDL-cholesterol (0.86 ± 0.16 vs. 1.19 ± 0.24 mmol/l; $P = 0.0009$).

TABLE 2. Fasting lipid/lipoprotein profiles of subjects according to status

Profile	Controls	Diabetics	*P*
Plasma			
C (mmol/l)	5.25 ± 1.16	6.11 ± 1.13	0.12
TG (mmol/l)	1.23 ± 0.67	4.60 ± 1.73	<0.0001
ApoB (g/l)	1.04 ± 0.28	1.26 ± 0.25	0.06
TRL			
C (mmol/l)	0.49 ± 0.32	2.12 ± 0.97	<0.0001
TG (mmol/l)	0.79 ± 0.57	4.17 ± 1.52	<0.0001
ApoB (g/l)	0.18 ± 0.07	0.27 ± 0.07	0.005
LDL			
C (mmol/l)	3.52 ± 1.05	3.15 ± 1.35	0.46
ApoB (g/l)	0.92 ± 0.27	0.88 ± 0.33	0.74
HDL			
C (mmol/l)	1.19 ± 0.24	0.86 ± 0.16	0.0009
Ratios			
TC/HDL-C	5.33 ± 2.87	7.10 ± 1.73	0.08
LDL-C/HDL-C	3.68 ± 2.21	3.57 ± 0.98	0.88

C, cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

TABLE 3. Kinetics of TRL apoB-48 in controls and type 2 diabetic patients

	TRL			
Subject	ApoB-48	PS	FCR	PR
	mg/dl	*mg*	*pools/day*	*mg/kg/day*
Controls (n = 13)	0.9 ± 0.6	32 ± 23	7.8 ± 2.0	3.0 ± 1.9
Diabetics (n = 11)	4.2 ± 2.0	162 ± 65	5.8 ± 1.6	11.0 ± 5.2
P	<0.0001	<0.0001	0.01	<0.0001

FCR, fractional catabolic rate; PR, production rate; PS, pool size.

Kinetics of TRL apoB-48 and apoB-100

Table 3 shows the kinetics of TRL apoB-48 according to status. Diabetic subjects had higher plasma apoB-48 concentrations than controls (4.2 ± 2.0 vs. 0.9 ± 0.6 mg/dl; $P < 0.0001$). Compared with controls, TRL apoB-48 PS was also increased in diabetic subjects (162 ± 65 vs. 32 ± 23 mg; $P < 0.0001$) as a result of an increase in TRL apoB-48 PR (11.0 ± 5.2 vs. 3.0 ± 1.9 mg/kg/day; $P < 0.0001$) and a decrease in TRL apoB-48 FCR (5.8 ± 1.6 vs. 7.8 ± 2.0 pools/day; $P = 0.01$) in these subjects. Multiple linear regression analysis was performed to assess the independent contribution of different metabolic and anthropometric variables to the variance of TRL apoB-48 PR. Age, BMI, and diabetic/control status were included in the model. As shown in **Table 4**, the diabetic/control status represented nearly 35% of the variance in TRL apoB-48 PR, whereas age contributed 26.7% to the variance of TRL apoB-48 PR. After adjustment for age and diabetic/control status, BMI was not found to significantly modulate TRL apoB-48 PR.

Table 5 shows the kinetics of apoB-100 according to status. Diabetics had higher VLDL apoB-100 PS (895 ± 243 vs. 321 ± 142 mg; $P < 0.0001$) as a result of a decreased VLDL apoB-100 FCR (4.1 ± 1.3 vs. 9.1 ± 3.4 pools/day; $P = 0.0002$) and a tendency toward an increased VLDL apoB-100 PR (41.1 ± 15.8 vs. 32.1 ± 7.7 mg/kg/day; $P = 0.08$). Similarly, IDL apoB-100 PS was increased in diabetic subjects (187 ± 62 vs. 33 ± 21 mg; $P < 0.0001$) as a result of a decreased IDL apoB-100 FCR (3.5 ± 1.2 vs. 7.3 ± 2.7 pools/day; $P = 0.0003$) and an increased IDL apoB-100 PR (6.7 ± 2.7 vs. 2.8 ± 1.6 mg/kg/day; $P = 0.0002$). No significant difference in LDL apoB-100 PS was seen between the two groups attributable to the concomitant increase in both LDL apoB-100 FCR and PR in diabetics. Only increased LDL apoB-100 PR, however,

TABLE 4. Multiple linear regression analysis showing independent contributions of age, BMI, and diabetic/control status to the variance of TRL apoB-48 PR

Independent Variable	Partial ($R^2 \times 100$)	*P*
Diabetic/control status	34.7	0.02
Age	26.7	0.03
BMI	3.3	NS

remained significant after adjustment for age and BMI. TRL apoB-48 PS was correlated with apoB-48 PR in controls ($r = 0.82$, $P = 0.0005$) and in diabetics ($r = 0.81$, $P = 0.003$). No significant correlation was found between TRL apoB-48 PS and FCR. In both groups, VLDL apoB-100 PS was correlated with FCR (controls, $r = -0.82$, $P = 0.0005$; diabetics, $r = -0.62$, $P = 0.04$) but not with PR (controls, $r = 0.09$, $P = 0.76$; diabetics, $r = 0.16$, $P = 0.63$). TRL apoB-48 PS and PR were correlated inversely with VLDL apoB-100 FCR (PS, $r = -0.88$, $P < 0.0001$; PR, $r = -0.77$, $P = 0.002$) in controls but not in diabetics (PS, $r = -0.35$, $P = 0.28$; PR, $r = -0.24$, $P = 0.48$). **Figure 1** illustrates the correlation between plasma TG levels and TRL apoB-48 PR and VLDL apoB-100 FCR in diabetic subjects. No significant correlation was found between TRL apoB-48 FCR and direct catabolism of IDL apoB-100 in both groups. Finally, there was no significant difference between controls and diabetics in either VLDL-to-IDL (5.1 ± 4.1 vs. 3.7 ± 1.6 pools/day; $P = 0.3$) or IDL-to-LDL (1.5 ± 2.4 vs. 0.5 ± 0.4 pools/day; $P = 0.2$) transfer rate.

DISCUSSION

This study provides information on the relationships between TRL apoB-48 and VLDL, IDL, and LDL apoB-100 kinetics in 11 type 2 diabetic males with marked increases in plasma TG levels and 13 nondiabetic controls. Specifically, we have demonstrated that these diabetic men had higher plasma concentrations and PR as well as lower FCR of intestinally derived apoB-48-containing lipoproteins than control individuals with lower insulin levels and greater insulin sensitivity. Importantly, multiple linear regression analyses revealed that the effect of diabetes on TRL apoB-48 PR and FCR remained independent of BMI and age. Our results indicate that the higher BMI in the diabetic group did not contribute significantly to the observed variations in TRL apoB-48 PR and FCR. Our findings support and extend the results of a recent study by Duez et al. (11) showing a positive correlation between TRL apoB-48 PR and fasting insulin concentrations in nondiabetic insulin-resistant subjects. Therefore, these results provide new evidence that diabetic dyslipidemia could be associated with overproduction and reduced clearance of both intestinally derived apoB-48- and TRL apoB-100-containing lipoproteins.

Previous studies have shown that both chylomicrons and VLDL increase after the ingestion of a fat-rich meal (20, 21). It is also well established that diabetic individuals have high levels of TRLs, including apoB-48-containing TRLs, in both the fasted and postprandial states (1, 22). The data reported here showing that TRL apoB-48 PS is correlated with TRL apoB-48 PR and not FCR indicate that the increase in apoB-48-containing lipoproteins is attributable to increased production. These results are in agreement with and extend previous findings by Duez et al. (11) showing that intestinal secretion of apoB-48-containing lipoproteins is increased in insulin-resistant humans with

TABLE 5. Kinetics of TRL, IDL, and LDL apoB-100 in controls and type 2 diabetic patients

Subject	VLDL				IDL				LDL			
	ApoB-100	PS	FCR	PR	ApoB-100	PS	FCR	PR	ApoB-100	PS	FCR	PR
	mg/dl	*mg*	*pools/day*	*mg/kg/day*	*mg/dl*	*mg*	*pools/day*	*mg/kg/day*	*mg/dl*	*mg*	*pools/day*	*mg/kg/day*
Controls (n = 13)	8.7 ± 3.2	321 ± 142	9.1 ± 3.4	32.1 ± 7.7	0.9 ± 0.6	33 ± 21	7.3 ± 2.7	2.8 ± 1.6	91.6 ± 22.9	3,333 ± 987	0.3 ± 0.1	12.9 ± 5.8
Diabetics (n = 11)	23.6 ± 8.2	895 ± 243	4.1 ± 1.3	41.1 ± 15.8	4.8 ± 1.6	178 ± 62	3.5 ± 1.2	6.7 ± 2.7	107.3 ± 52.4	4,271 ± 2,393	0.6 ± 0.3	23.0 ± 11.8
P	<0.0001	<0.0001	0.0002	0.08	<0.0001	<0.0001	0.0003	0.0002	0.34	0.21	0.04	0.01

IDL, intermediate density lipoprotein.

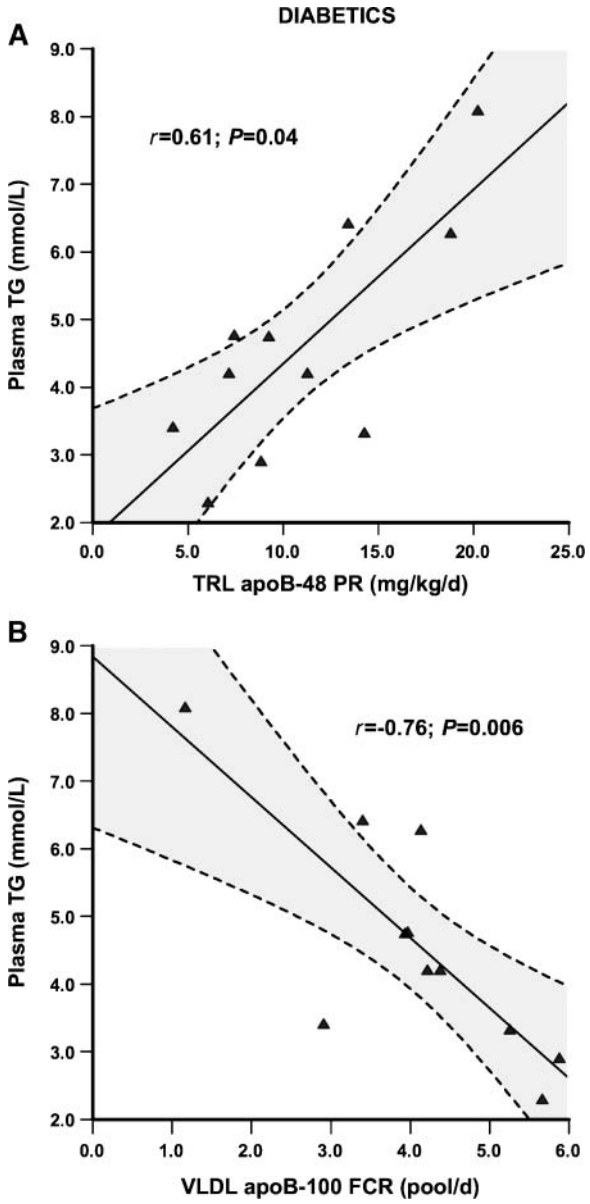

Fig. 1. Correlation between plasma triglyceride (TG) levels and triglyceride-rich lipoprotein apolipoprotein B-48 (TRL apoB-48) production rate (PR) (A) and VLDL apoB-100 fractional catabolic rate (FCR) (B) in patients with type 2 diabetes.

hyperinsulinemia. Our study was not designed to address the cellular mechanisms underlying the increased production of apoB-48-containing lipoproteins by the intestine in individuals with type 2 diabetes. However, recent studies using cultured primary enterocytes from fructose-fed insulin-resistant hamsters have demonstrated an increased secretion of apoB-48-containing lipoproteins accompanied by increased intracellular apoB-48 stability, enhanced de novo lipid synthesis, and higher abundance of microsomal TG transfer protein mass (23, 24). Therefore, it is likely that enhanced intestinal lipogenesis may play an important role in increasing the secretion of apoB-48-containing lipoproteins by the intestine in subjects with type 2 diabetes. Several lines of evidence also indicate that increased FFA flux from adipose tissue to the liver stimulates the hepatic assembly and secretion of apoB-100-containing lipoproteins in insulin-resistant subjects (25, 26). Similarly, recent studies using hamster enterocytes in primary culture showed that oleic acid stimulated the secretion of apoB-48-containing lipoproteins (27) and that intestinal lipoprotein production was markedly increased by an acute increase of plasma FFA in hamsters (28). Further studies are required to assess the potential correlation between increased FFA flux from adipose tissue to the liver and the intestinal production of TRL apoB-48 in humans.

As reported previously (16), this study also indicates that VLDL apoB-100 PS is correlated with the catabolism of VLDL apoB-100 and not PR in both controls and diabetics. Consequently, the increase in VLDL apoB-100 after the ingestion of a fat-rich meal is related to decreased catabolism of VLDL apoB-100. Modest reductions in postheparin LPL activity have been reported in patients with type 2 diabetes (22), and this may contribute significantly to decreased TRL catabolism. In addition, our study shows an inverse correlation between TRL apoB-48 PS and PR and VLDL apoB-100 FCR in controls with relatively low TG levels, a finding supported by previous observations in humans (16, 29). However, the absence of correlation between TRL apoB-48 PS and PR and VLDL apoB-100 FCR in diabetics with severe hypertriglyceridemia suggests that the competition between TRL apoB-48 and VLDL apoB-100 for the same catabolic pathway could be dependent on the number of circulating TRLs. It is likely that high plasma concentrations of TRLs could saturate the catabolic pathway and greatly reduce the capacity of apoB-48-containing lipoproteins to compete with TRL apoB-100 for catabolism. Furthermore, because both IDL and chylomicron remnants are presumably cleared by the same catabolic pathway, one might expect a direct correlation between FCRs of TRL apoB-48 and IDL apoB-100. However, there was no significant correlation between TRL apoB-48 FCR and direct catabolism of IDL apoB-100 in both groups. These results are in agreement with findings of a previous study (16) examining correlations between apoB-48 and apoB-100 kinetics in control subjects and suggest the presence of significant differences between IDL and chylomicron remnant catabolism. Further animal and human studies examining factors involved in the regulation of IDL and chylomicron remnant catabolism are needed to clarify this issue.

In this study, we observed that LDL apoB-100 PR and FCR were higher in subjects with type 2 diabetes than in controls. Only differences in LDL apoB-100 PR, however, remained significant after adjustment for age and BMI. This finding contrasts with results of previous studies with type 2 diabetics showing both a decrease in LDL catabolism attributed to the glycation of apoB-100 (30) and a reduction in the expression of LDL receptor (31) as well as a trend toward reduced LDL synthesis (32). In this regard, it is likely that the increased LDL apoB-100 PR observed in diabetic individuals was attributable to increased VLDL and IDL apoB-100 PS associated with relatively normal

VLDL-to-IDL and IDL-to-LDL transfer rates. It is interesting that the difference in LDL apoB-100 PR between diabetics and controls is no longer significant after adjustment for VLDL apoB-100 PS or plasma TG levels. Therefore, further studies including diabetics and controls with comparable plasma TG levels are needed to clarify the influence of type 2 diabetes on LDL apoB-100 kinetics.

In this study, diabetic subjects were older and had higher BMI compared with controls, and we cannot exclude the possibility that these variables may have contributed to the observed increase in TRL apoB-48 PR or may have affected FCR. After adjustment for these two variables, however, type 2 diabetes was found to be independently associated with increased TRL apoB-48 secretion. Furthermore, it is important to emphasize that diabetic subjects included here are on average severely hypertriglyceridemic to an extent that is not typical of diabetic subjects, with glycated hemoglobin of <9%. Therefore, we cannot exclude the possibility that another factor, such as primary hyperlipidemia or some other secondary cause increasing plasma TG levels, is involved. Further studies with diabetic patients having more typical TG levels are needed to examine the correlation between plasma TG levels and TRL apoB-48 PR.

In conclusion, this study provides evidence that type 2 diabetes associated with severe hypertriglyceridemia is also associated with both increased production and decreased catabolism of intestinally derived apoB-48-containing lipoproteins. Additional studies are required to elucidate the molecular mechanisms responsible for the increased TRL apoB-48 PR in this condition and to assess the contribution of these lipoproteins to the development of atherosclerosis in these patients.

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