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Differential effect of fenofibrate and atorvastatin on in vivo kinetics of apolipoproteins B-100 and B-48 in subjects with type 2 diabetes mellitus with marked hypertriglyceridemia

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Abstract

The specific impact of 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors and fibrates on the in vivo metabolism of apolipoprotein (apo) B has not been systematically investigated in patients with type 2 diabetes mellitus with high plasma triglyceride (TG) levels. Therefore, the objective of this 2-group parallel study was to examine the differential effects of a 6-week treatment with atorvastatin or fenofibrate on in vivo kinetics of apo B-48 and B-100 in men with type 2 diabetes mellitus with marked hypertriglyceridemia. Apolipoprotein B kinetics were assessed at baseline and at the end of the intervention using a primed constant infusion of [5,5,5-D_3]-L-leucine for 12 hours in the fed state. Fenofibrate significantly decreased plasma TG levels with no significant change in plasma low-density lipoprotein cholesterol (LDL-C) and apo B levels. On the other hand, atorvastatin significantly reduced plasma levels of TG, LDL-C, and apo B. After treatment with fenofibrate, very low-density lipoprotein (VLDL) apo B-100 pool size (PS) was decreased because of an increase in the fractional catabolic rate (FCR) of VLDL apo B-100. No significant change was observed in the kinetics of LDL apo B-100. Moreover, fenofibrate significantly decreased TG-rich lipoprotein (TRL) apo B-48 PS because of a significant increase in TRL apo B-48 FCR. After treatment with atorvastatin, VLDL and IDL apo B-100 PSs were significantly decreased because of significant elevations in the FCR of these subfractions. Low-density lipoprotein apo B-100 PS was significantly lowered because of a tendency toward decreased LDL apo B-100 production rate (PR). Finally, atorvastatin reduced TRL apo B-48 PS because of a significant decrease in the PR of this subfraction. These results indicate that fenofibrate increases TRL apo B-48 as well as VLDL apo B-100 clearance in men with type 2 diabetes mellitus with marked hypertriglyceridemia, whereas atorvastatin increases both VLDL and IDL apo B-100 clearance and decreases TRL apo B-48 and LDL apo B-100 PR.

1. Introduction

Diabetes mellitus is a complex and multifactorial disease known to induce important lipoprotein abnormalities and to increase the risk of cardiovascular disease by 2 to 3 times [1]. These atherogenic abnormalities are related to both qualitative and quantitative disturbances in triglyceride (TG)-rich lipoprotein (TRL) metabolism and include elevated plasma levels of TG due to an increased hepatic production of atherogenic very low-density lipoprotein (VLDL); low high-density lipoprotein cholesterol (HDL-C); and increased numbers of small, cholesteryl ester–depleted, dense low-density lipoprotein (LDL) [2,3].

Lifestyle modifications are the cornerstone of the treatment of type 2 diabetes mellitus and its associated lipoprotein abnormalities. However, in most diabetic patients, lipid-modifying drugs are required to achieve significant improvement of the lipoprotein profile. Fibrates are peroxisome proliferator–activated receptor (PPAR) α agonists that have been shown to reduce plasma concentrations of atherogenic TRL, to increase HDL-C levels, as well as to reduce LDL density [4]. In some patients, however, an

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unwanted raise in LDL cholesterol (LDL-C) and apo B levels may occur, this effect being more prevalent in patients having high plasma TG levels at baseline [5,6]. On the other hand, 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) have been shown to exert their beneficial effects on lipoprotein profile mainly by decreasing plasma concentrations of apo B–containing lipoproteins via LDL receptor up-regulation and by increasing HDL-C levels to some extent [7]. Kinetic studies performed in animals and in nondiabetic human subjects have also demonstrated that statins reduce hepatic secretion of VLDL [8]. In addition, treatment with statins has been associated with significant increases in LDL particle size [9-11].

Apolipoprotein B-100 is a large glycoprotein secreted by the liver and plays a central role for the assembly, secretion, and intravascular transport of VLDL, intermediate-density lipoprotein (IDL), and LDL. Apolipoprotein B-100 interacts with the LDL receptor responsible for approximately two thirds of LDL uptake. Apolipoprotein B-48 is the principal structural protein of intestinally derived lipoproteins and remains associated with them throughout the whole lipolytic cascade leading to chylomicron remnants. The hepatic clearance of apo B-48– and apo B-100–containing lipoproteins is mediated by the action of hepatic lipase, lipoprotein lipase (LPL), apo E, LDL receptor, LDL-receptor–related protein, and heparin sulfate proteoglycans [12]. Emerging data have recently contributed to highlight the important role of apo B-48–containing lipoproteins in atherogenesis [13-15], whereas the role of apo B-100–containing lipoproteins in this process is well established [16]. Despite the widespread use of fibrates and statins for the treatment of diabetic patients with dyslipidemia, the specific impact of these 2 distinct classes of lipid-lowering drugs on the in vivo metabolism of apo B-48 and B-100 has not been systematically investigated in patients with type 2 diabetes mellitus with high plasma TG levels. Therefore, the aim of the present study was to examine the impact of atorvastatin and fenofibrate on the kinetics of apo B-48 and B-100 in subjects with type 2 diabetes mellitus with marked hypertriglyceridemia.

2. Methods

2.1. Subjects

Eleven men with type 2 diabetes mellitus and marked hypertriglyceridemia (range, 2.3-8.1 mmol/L) were included in this study. Subjects had to have type 2 diabetes mellitus as defined by the American Diabetes Association [17] and to receive stable doses of oral hypoglycemic drugs for at least 3 months before the study to achieve glycosylated hemoglobin (HbA_{1C}) values less than 9%. All patients were treated with either metformin or a combination of metformin and a sulfonylurea. Three subjects received a thiazolidinedione (TZD). All subjects had to be withdrawn from lipid-lowering medications for at least 6 weeks before the kinetic study in the basal state. Exclusion criteria include personal history of cardiovascular disease; microalbuminuria; genetic condition affecting lipid metabolism (eg, familial hypercholesterolemia, type III hyperlipidemia, LPL deficiency, etc); body mass index (BMI) <25.0 or >35.0 kg/m^2; uncontrolled hypothyroidism; nephrotic syndrome; anorexia nervosa; hypersensitivity to statins or fibrates; history of alcohol or drug abuse; persistent elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), or creatine phosphokinase; uncontrolled endocrine or metabolic disease; uncontrolled diabetes mellitus (HbA_{1c} >9%); poor mental condition; or a positive test result for HIV. Upon their entry into the study, subjects met with a dietitian 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 2 groups. The research protocol was approved by the Laval University Medical Center ethical review committee, and written informed consent was obtained from each subject.

2.2. Study design and experimental protocol for in vivo stable isotope kinetics

After a 6-week washout period, patients were randomized and blindly (double-blind) assigned to receive either atorvastatin 20 mg/d or micronized fenofibrate 200 mg/d for 6 weeks. Each subject underwent a kinetic study before and after the treatment period. Patients were instructed to take one capsule at the time of their evening meal. Compliance was assessed by pill counting.

To determine the kinetics of TRL apo B-48, VLDL, IDL, and LDL apo B-100, subjects underwent a primed constant infusion of L-[5,5,5-D_3]leucine while they were in a constantly fed state. Starting at 7:00 AM, the subjects received 30 identical small cookies every half hour for 15 hours, each equivalent to 1/30th of their estimated daily food intake based on the Harris-Benedict equation [18] with 15% of calories as protein, 45% carbohydrate, and 40% fat (7% saturated, 26% monounsaturated, 7% polyunsaturated), and 85 mg of cholesterol per 1000 kcal. At 10:00 AM, with 2 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 intravenously as a bolus and then by continuous infusion (10 μmol per kilogram body weight per hour) over a 12-hour period. Blood samples (20 mL) were collected at hours 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12.

2.3. Characterization of fasting plasma lipids and lipoproteins

Before the kinetic studies, 12-hour fasting venous blood samples were obtained from antecubital vein into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA (0.1% final concentration). Samples were then immediately centrifuged at 4°C for 10 minutes at 3000

rpm to obtain plasma and were stored at 4°C until processed. Plasma VLDL (density <1.006 g/mL) was isolated by preparative ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant (density >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. Apolipoprotein B 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. High-density lipoprotein apo A-I levels were measured by nephelometry (Dade Behring).

2.4. Measurement of LPL activity

Plasma LPL activity was measured in each subject 10 minutes after the intravenous injection of heparin (60 IU/kg of body weight). Lipoprotein lipase activity is expressed as micromoles of fatty acids released per milliliter of plasma per hour [19].

2.5. Quantification and isolation of apo B-48 and B-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. Apolipoprotein B concentration in TRL, IDL, and LDL were determined by noncompetitive enzyme-linked immunosorbent assay using immunopurified polyclonal antibodies (Alerchek, Portland, ME) to calculate their respective pool size (PS). The coefficient of variation for the apo B assay was between 6% and 10% depending upon the region of the standard curve. Apolipoprotein B-48 was assessed in TRL (VLDL) fraction only. Apolipoprotein B-100 and apo B-48 were separated by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis according to standardized procedures [20]. Briefly, 50 μL of each fraction was mixed with 50 μL of 3% sodium dodecyl sulfate sample buffer and subjected to electrophoresis in 3% to 10% linear gradient polyacrylamide slab mini gels. Gels were stained overnight in 0.25% Coomassie blue R-250, destained for 7 to 8 hours. Based on the assumption that both apo B-100 and apo B-48 have the same chromogenicity, the relative proportion of apo B-100 and apo B-48 was assessed by scanning each gel with laser densitometry [21]. We scanned lipoprotein fractions from 3 different time points to calculate ratios and to estimate the average concentrations of apo B-100 and apo B-48 using the total apo B concentration.

2.6. Isotopic enrichment determinations

Apolipoprotein B-48 and apo B-100 bands were excised from polyacrylamide gels, and bands were hydrolyzed in 6 N HCl at 110°C for 24 hours [22]. Trifluoroacetic acid and trifluoroacetic anhydride (1:1) were used as derivatization reagents for the amino acids before analysis on a Hewlett-Packard (Santa Clara, CA) 6890/5973 gas chromatograph/mass spectrometer [23]. Isotope enrichment (percentage) and tracer-tracee ratio (percentage) were calculated from the observed ion current ratios [24]. The isotopic enrichment of leucine in the apolipoproteins was expressed as tracer-tracee ratio (percentage) using standarized formulas [24].

2.7. Kinetic analysis

Kinetics of apo B-48 and apo B-100 were derived by a multicompartmental model as previously described [25]. We assumed a constant enrichment of the precursor pool and used either the TRL apo B-48 or the VLDL apo B-100 plateau tracer-tracee ratio data as the forcing function to drive the appearance of tracer into apo B-48 and apo B-100, respectively [22]. Under steady-state condition, the fractional catabolic rate (FCR) is equivalent to the fractional synthetic rate. Apolipoprotein B production rates (PRs) were determined by the formula PR (milligrams per kilogram per day) = [FCR (pools per day) × apo B concentration (milligrams per liter) × plasma volume (liters)]/body weight (kilograms) [26]. 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.

2.8. Statistical analysis

Data from the 2 groups were compared using χ^2 tests for categorical measures and analysis of variance tests for continuous variables with normal distribution. Paired *t* tests were used to compare continuous variables within each group. 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 interpret the relationship of these associations. All analyses were performed using JMP Statistical Software (version 6.03; SAS Institute, Cary, NC).

3. Results

3.1. Demographic, anthropometric, and biochemical characteristics of subjects

Table 1 shows the demographic, anthropometric, and biochemical characteristics of the participants in the fasting state before and after treatment with either fenofibrate 200 mg/d (n = 5) or atorvastatin 20 mg/d (n = 6). There was no significant difference in age, BMI, HbA_{1C}, fasting glycemia, 3-hour postprandial glycemia, ALT, AST, LPL activity, and baseline plasma lipid/lipoprotein profile between the 2 groups. Treatment with fenofibrate significantly reduced plasma TG (−39.4%, P = .03), TRL cholesterol (−49.7%, P = .01), and TRL-TG (−47.1%, P = .03) and significantly increased LPL activity (+49.0%, P < .0001). Treatment with atorvastatin significantly decreased plasma C (−30.7%, P = .0003), plasma TG (−31.4%, P =

Table 1
Characteristics and lipid/lipoprotein profile of patients with type 2 diabetes mellitus with marked hypertriglyceridemia before and after treatment with fenofibrate 200 mg/d or atorvastatin 20 mg/d

	Fenofibrate treatment (n = 5)				Atorvastatin treatment (n = 6)				*P* between baseline	*P* between %Δ
	Baseline	Fenofibrate	%Δ	*P*	Baseline	Atorvastatin	%Δ	*P*		
Age (y)	55.1 ± 4.5	–	–	–	53.2 ± 9.7	–	–	–	.70	–
BMI (kg/m^2)	29.3 ± 3.7	–	–	–	29.4 ± 4.0	–	–	–	.95	–
HbA_{1C} (%)	7.4 ± 1.0	7.2 ± 0.7	−2.7	.34	7.4 ± 1.1	7.3 ± 1.3	−1.4	.11	.95	.78
Fasting glucose (mmol/L)	9.1 ± 2.6	7.9 ± 2.0	−13.8	.02	8.3 ± 1.5	8.1 ± 1.5	−3.2	.22	.55	.09
Postprandial (3 h) glucose (mmol/L)	10.0 ± 2.6	11.2 ± 3.0	+11.8	.15	9.1 ± 2.6	9.5 ± 2.5	+4.2	.24	.57	.48
ALT (U/L)	20 ± 3	16 ± 7	−20.0	.23	23 ± 15	25 ± 13	+8.7	.22	.62	.17
AST (U/L)	22 ± 2	22 ± 2	0.0	.77	26 ± 5	26 ± 5	0.0	.91	.14	.70
LPL activity (μU [mL h])	3.1 ± 1.4	4.6 ± 2.0	+49.0	<.0001	2.7 ± 1.3	2.6 ± 1.1	−1.5	.43	.44	.01
Metformin (n)	4				5					
Glyburide (n)	1				1					
Gliclazide (n)	2				1					
Rosiglitazone (n)	1				2					
Plasma										
C (mmol/L)	5.72 ± 0.91	5.15 ± 1.03	−10.0	.06	6.25 ± 1.32	4.33 ± 0.82	−30.7	.0003	.47	.004
TG (mmol/L)	4.04 ± 1.59	2.45 ± 1.01	−39.4	.03	5.07 ± 1.83	3.48 ± 1.05	−31.4	.01	.35	.53
Apo B (g/L)	1.18 ± 0.25	1.09 ± 0.30	−7.6	.17	1.32 ± 0.26	0.78 ± 0.36	−40.9	.004	.38	.02
TRL										
C (mmol/L)	1.81 ± 0.69	0.91 ± 0.49	−49.7	.01	2.26 ± 1.13	1.55 ± 0.77	−31.4	.02	.46	.17
TG (mmol/L)	3.80 ± 1.57	2.01 ± 0.98	−47.1	.02	4.25 ± 1.50	2.55 ± 1.55	−40.0	.03	.64	.73
Apo B (g/L)	0.22 ± 0.02	0.21 ± 0.01	−4.6	.19	0.29 ± 0.07	0.30 ± 0.15	+3.5	.44	.07	.74
LDL										
C (mmol/L)	3.17 ± 1.52	3.29 ± 1.06	+3.4	.42	2.61 ± 0.99	1.46 ± 0.42	−44.1	.004	.48	.02
TG (mmol/L)	0.30 ± 0.13	0.30 ± 0.11	0.0	.50	0.28 ± 0.07	0.68 ± 1.19	+142.9	.22	.70	.48
Apo B (g/L)	0.88 ± 0.38	0.90 ± 0.37	+2.3	.45	0.74 ± 0.24	0.40 ± 0.11	−46.0	.002	.46	.03
HDL										
C (mmol/L)	0.77 ± 0.20	0.86 ± 0.21	+11.7	.11	0.73 ± 0.07	0.89 ± 0.09	+21.9	.02	.64	.45
Apo A-I (g/L)	0.73 ± 0.25	0.77 ± 0.19	+5.5	.18	0.64 ± 0.10	0.78 ± 0.19	+21.9	.02	.42	.31

%Δ represents the percentage of change between baseline and treated values.

.01), plasma apo B (−40.9%, *P* = .004), TRL cholesterol (−31.4%, *P* = .02), TRL-TG (−40.0%, *P* = .03), LDL-C (−44.1%, *P* = .004), and LDL apo B (−46.0%, *P* = .002). Moreover, atorvastatin treatment significantly increased HDL-C (+21.9%, *P* = .02) and HDL apo A-I (+21.9%, *P* = .02). Finally, significant differences in the percentages of response between the 2 treatment groups were observed for plasma apo B (*P* = .02), LDL-C (*P* = .02), and LDL apo B levels (*P* = .03). There was no significant difference in plasma lipid levels between patients receiving TZD treatment and those not on TZD (data not shown).

3.2. Kinetics of TRL apo B-48

Analyses of deuterated plasma amino acids and lipid/lipoprotein measurements indicated that plasma leucine enrichments as well as plasma TG and TRL apo B-48 levels remained constant during the kinetic study (data not shown). The mean tracer-tracee ratio curves for apo B-48 at baseline and after treatment are illustrated in Fig. 1. Table 2 shows the nonfasting TRL apo B-48 concentrations, PS, FCR, and PR for the 2 treatment groups. No difference was observed at baseline between the 2 groups. Fenofibrate treatment was associated with a significant reduction in TRL apo B-48 concentrations (−37.1%, *P* = .02) and PS (−36.4%, *P* = .02) due to an increase in TRL apo B-48 FCR (+25.0%, *P* = .02). Fenofibrate also increased the transfer rates of VLDL to IDL and of IDL to LDL by 15% and 10%, respectively. On the other hand, atorvastatin decreased TRL apo B-48 concentrations and PS by −36.7% and −33.9%, respectively; but these changes did not reach statistical significance. The reduction in the PR of TRL apo B-48 with atorvastatin, however, did reach statistical significance (−36.5%, *P* = .04). Finally, atorvastatin increased VLDL to IDL transfer rate by 45% but was associated with an 18% reduction in IDL to LDL transfer rate.

3.3. Kinetics of VLDL, IDL, and LDL apo B-100

Table 3 shows the nonfasting VLDL, IDL, and LDL apo B-100 concentrations, PS, FCR, and PR for the 2 treatment groups. The mean tracer-tracee ratio curves for apo B-100 at baseline and after treatment with either fenofibrate or atorvastatin are shown in Fig. 2. No significant difference in kinetic variables was observed between the 2 groups at baseline. Treatment with fenofibrate significantly increased VLDL apo B-100 FCR (+42.9%, *P* = .007) and tended to increase IDL apo B-100 FCR (+30.6%, *P* = .07). Fenofibrate also tended to reduce VLDL apo B-100 concentrations (−23.8%, *P* = .06) and VLDL apo B-100 PS (−23.9%, *P* = .06). After treatment with atorvastatin, VLDL and IDL apo B-100 PSs were significantly decreased by −30.3% and

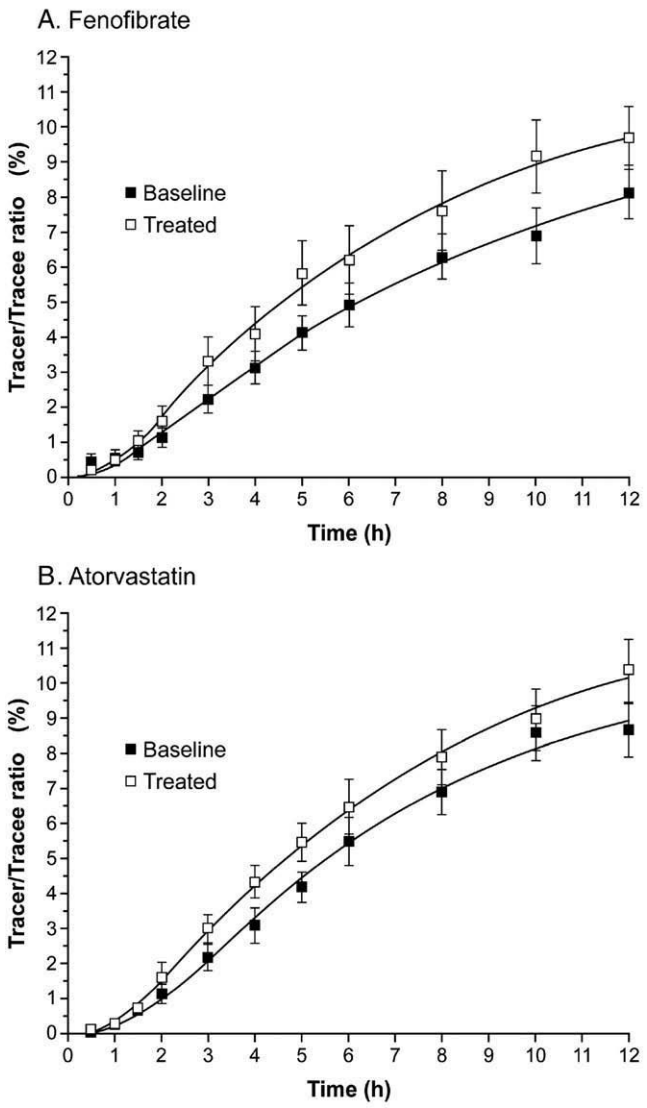

Fig. 1. Apolipoprotein B-48 leucine tracer-tracee ratios for TRL in patients with type 2 diabetes mellitus with marked hypertriglyceridemia before and after treatment with either fenofibrate 200 mg/d (A) or atorvastatin 20 mg/d (B).

−18.2% ($P < .05$), respectively, because of significant increases in the FCR of these subfractions (+25.0%, $P = .02$ and +45.5%, $P = .0006$, respectively). Furthermore, LDL apo B-100 PS was significantly decreased by −47.8% ($P = .03$) because of a tendency toward a reduction in PR (−44.4%, $P = .09$).

4. Discussion

The present study provides new insight on the effects of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor atorvastatin in diabetic subjects with hypertriglyceridemia and compares its action with those of fenofibrate, a PPARα agonist. Dyslipidemia associated with type 2 diabetes mellitus is typically characterized by hypertriglyceridemia, reduced HDL-C levels, and increased proportion of small dense LDL particles [2,3]. It is now well established that hypertriglyceridemia in type 2 diabetes mellitus is attributed to fasting and postprandial overaccumulation of apo B-100–containing TRL and intestinally derived apo B-48–containing lipoproteins [27,28]. In fact, several lines of evidence indicate that insulin resistance is associated with elevated apo B-100–containing TRL secretion by the liver, with a significant contribution from reduced VLDL clearance [29]. Similarly, overaccumulation of apo B-48–containing lipoproteins associated with insulin resistance has been attributed to several factors, namely, impaired clearance of TRL remnants due to competition for removal between apo B-100– and apo B-48–containing lipoproteins, modified TRL apolipoprotein composition, as well as decreased LPL activity [30,31]. Recent studies [32,33] also suggested that overaccumulation of apo B-48–containing lipoproteins in insulin-resistant humans could result to some extent from an increased intestinal lipoprotein PR. In the present study, it is interesting to note that the plasma apo B-48 PS was approximately 7-fold higher than that of insulin-resistant subjects [32] or controls [22]. Such variations in TRL apo B-48 PS are most likely explained by major differences in baseline plasma TG levels and by the fact that our subjects received a high–fat content diet (40% fat) that is likely to induce a higher postprandial apo B-48 level than that induced by the diets provided by Duez et al [32] (18% fat) or Welty et al [22] (36% fat).

In the present study, the significant reductions in LDL-C and LDL apo B-100 by atorvastatin were associated with a tendency toward decreased production of LDL apo B-100; but, unexpectedly, no change was observed in the FCR of this subfraction. Most studies have shown that statins decrease plasma LDL-C by up-regulating LDL receptor gene and hence increasing catabolism of LDL apo B-100 [34-36]. Our results, however, do not support this mechanism. In accordance, Myerson et al [37] reported that

Table 2
Kinetics of TRL apo B-48 in patients with type 2 diabetes mellitus with marked hypertriglyceridemia before and after treatment with either fenofibrate 200 mg/d or atorvastatin 20 mg/d

		TRL			
		Apo B-48 (mg/dL)	PS (mg)	FCR (pools/d)	PR (mg/[kg·d])
Fenofibrate treatment	Baseline	3.5 ± 0.5	132 ± 27	5.6 ± 2.2	9.1 ± 4.5
	Fenofibrate	2.2 ± 0.7	84 ± 33	7.0 ± 1.7	6.8 ± 2.5
	%Δ	−37.1	−36.4	+25.0	−25.3
	P	.02	.02	.02	.09
Atorvastatin treatment	Baseline	4.9 ± 2.5	186 ± 80	5.9 ± 0.9	12.6 ± 5.5
	Atorvastatin	3.1 ± 0.7	123 ± 38	5.9 ± 1.4	8.0 ± 1.6
	%Δ	−36.7	−33.9	0.0	−36.5
	P	.06	.06	.50	.04
P between baseline		.27	.19	.73	.28
P between %Δ		.58	.58	.06	.56

%Δ represents the percentage of change between baseline and treated values.

Table 3
Kinetics of VLDL, IDL, and LDL apo B-100 in patients with type 2 diabetes mellitus with marked hypertriglyceridemia before and after treatment with either fenofibrate 200 mg/d or atorvastatin 20 mg/d

		VLDL				IDL				LDL			
		Apo B-100 (mg/dL)	PS (mg)	FCR (pools/d)	PR (mg/[kg·d])	Apo B-100 (mg/dL)	PS (mg)	FCR (pools/d)	PR (mg/[kg·d])	Apo B-100 (mg/dL)	PS (mg)	FCR (pools/d)	PR (mg/[kg·d])
Fenofibrate treatment	Baseline	21 ± 6.4	803 ± 107	4.2 ± 1.2	39 ± 8.8	3.6 ± 1.3	140 ± 54	3.6 ± 1.3	5.5 ± 1.7	112 ± 68	4268 ± 2790	0.4 ± 0.2	18. ± 5.3
	Fenofibrate	16 ± 4.7	611 ± 209	6.0 ± 0.9	42 ± 9.9	4.2 ± 1.0	159 ± 60	4.7 ± 2.1	8.7 ± 6.2	103 ± 51	3978 ± 2185	0.5 ± 0.3	22 ± 13
	%Δ	−23.8	−23.9	+42.9	+7.7	+16.7	+13.6	+30.6	+58.2	−8.0	−6.8	+25.0	+22.2
	P	0.06	0.06	0.007	0.28	0.30	0.30	0.07	0.17	0.35	0.36	0.26	0.32
Atorvastatin treatment	Baseline	25 ± 9.7	971 ± 261	4.0 ± 1.5	43 ± 21	5.4 ± 1.4	209 ± 51	3.3 ± 1.2	7.7 ± 3.0	103 ± 42	4273 ± 2286	0.7 ± 0.4	27 ± 14
	Atorvastatin	17 ± 3.5	677 ± 147	5.0 ± 1.3	38 ± 10	4.4 ± 2.5	171 ± 90	4.8 ± 1.3	8.9 ± 5.1	56 ± 16	2230 ± 686	0.6 ± 0.3	15 ± 6.5
	%Δ	−32.0	−30.3	+25.0	−11.6	−18.5	−18.2	+45.5	+15.6	−45.6	−47.8	−14.3	−44.4
	P	.03	.02	.02	.20	.04	.04	.0006	.21	.02	.03	.52	.09
P between baseline		.46	.28	.74	.72	.07	.06	.70	.18	.81	.99	.27	.23
P between %Δ		.74	.74	.98	.69	.23	.23	.34	.47	.05	.05	.68	.50

%Δ represents the percentage change between baseline and treated values.

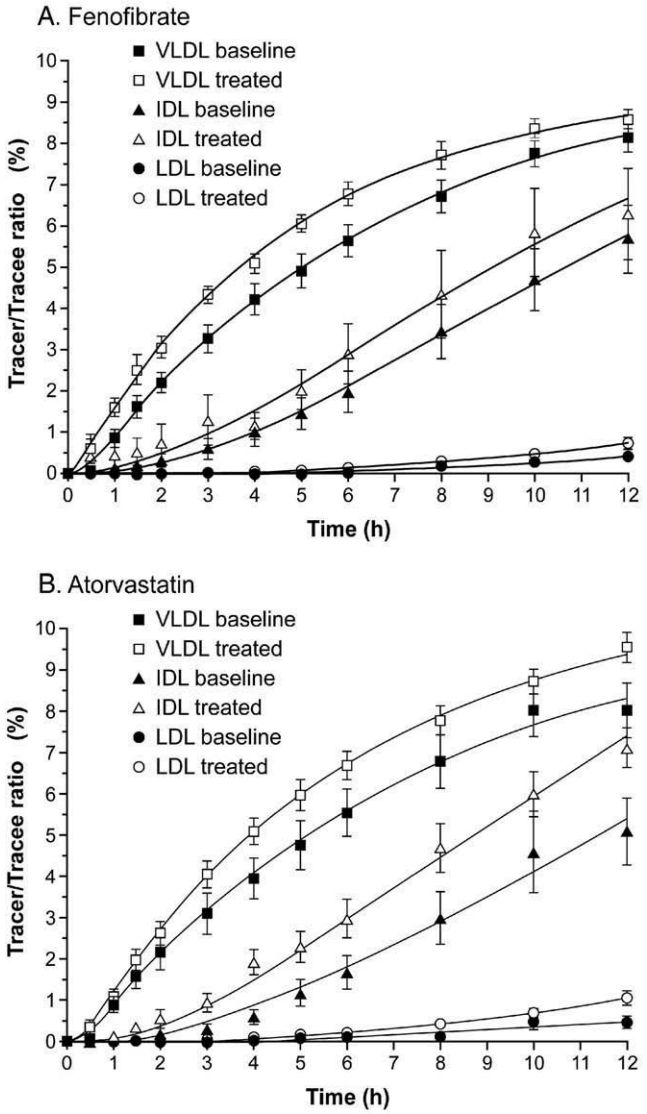

Fig. 2. Apolipoprotein B-100 leucine tracer-tracee ratios for VLDL, IDL, and LDL in patients with type 2 diabetes mellitus with marked hypertriglyceridemia before and after treatment with either fenofibrate 200 mg/d (A) or atorvastatin 20 mg/d (B).

simvastatin had no effect on the FCR of LDL apo B-100 in patients with type 2 diabetes mellitus and suggested that statins may increase LDL apo B-100 FCR in individuals with low baseline LDL apo B-100 FCR. In our study, the LDL apo B-100 PR after treatment with atorvastatin was inversely correlated with baseline status ($r = -0.82$, $P = .04$), suggesting that atorvastatin was most effective in subjects with elevated LDL apo B-100 PR at baseline. Therefore, these discrepancies could be explained by different baseline metabolic conditions in our patients with type 2 diabetes mellitus and marked hypertriglyceridemia.

Statins have consistently been shown to decrease plasma TG levels. In the present study, atorvastatin decreased VLDL and IDL apo B-100 PS by increasing the clearance of these subfractions. This finding is in agreement with recently reported results from other apo B kinetic studies in both diabetic and nondiabetic subjects [36,38-40]. Because LDL apo B-100 FCR remained unchanged with atorvastatin, it can be hypothesized that the increased clearance of VLDL and IDL apo B-100 with atorvastatin was likely due to an up-regulation of LDL receptor–related protein or other receptors not specifically involved in LDL particle clearance. Another possible explanation would be that the increased catabolism of TRL after treatment with atorvastatin is at least in part secondary to an increase in LPL activity resulting from a decrease in apo C-III expression [41]. Martin et al [42] have recently suggested that the activation of PPARα would be implicated as a mediator of statin effects of lipoprotein metabolism. This mechanism, however, is less likely because LPL activity was unchanged after treatment with atorvastatin. Furthermore, our study showed that treatment with atorvastatin did not decrease significantly the secretion of VLDL apo B-100, a finding in agreement with some, but not all, reports of apo B kinetics in individuals with mixed hyperlipidemia [39,43,44]. As suggested by Chan et al [45], these discrepancies could be explained to some extent by the persistent effects of insulin resistance on the hepatic processing of lipids and apolipoproteins as well as by an associated increase in intestinal cholesterol absorption and cholesteryl ester delivery to the liver from the intestine [46] that could potentially override the inhibitory effects of atorvastatin on apo B secretion. The latter mechanism, however, does not appear to play a major role in the present study because treatment with atorvastatin was associated with a concomitant reduction in TRL apo B-48 secretion rate, suggesting that lipid supply from the intestine to the liver was in fact reduced by atorvastatin. The reduction in TRL apo B-48 secretion rate observed in the present study is in agreement with a recent study by Lally et al [47] showing important alterations in the expression of intestinal genes that regulate cholesterol absorption and chylomicron synthesis in subjects with type 2 diabetes mellitus. Lally et al also observed that treatment of patients with type 2 diabetes mellitus with simvastatin was associated with reduced expression of microsomal TG transfer protein, an enzyme playing a crucial role in chylomicron synthesis, as well as increased messenger RNA levels of ABCG5 and ABCG8, which are known to promote active efflux of cholesterol from enterocytes back into the intestinal lumen for excretion. Our results are also consistent with a previous report [48] showing that atorvastatin reduces plasma concentrations of apo B-48 but contrast with a study by Watts and colleagues [49] relating this effect to an accelerated FCR of chylomicron remnants, as measured by a stable isotope breath test. Further studies are clearly needed to clarify the impact of statins on apo B-48 kinetics in hypertriglyceridemic humans.

Fibrates have been shown to decrease plasma TG and VLDL-C levels and to increase HDL-C as well as LDL particle size, thereby favorably influencing the lipoprotein profile. However, the response of hypertriglyceridemic patients to fibrate treatment is heterogenous; and

nonresponse or even paradoxical increase in LDL-C has been observed. In fact, the enhanced catabolism of TRL induced by fibrates has been shown to increase plasma levels of LDL-C in a large proportion of hypertriglyceridemic patients [50]. The beneficial effects of fibrates on lipoprotein profile result from the activation of the nuclear receptor PPARα and have been attributed to several mechanisms including (1) increased TRL lipolysis associated with increased intrinsic LPL activity or increased accessibility of TRL for lipolysis due to a reduction in TRL apo C-III content; (2) increased catabolism of LDL particles exhibiting a greater affinity for the LDL receptor; (3) induction of hepatic fatty acid uptake and reduction of hepatic TG production; (4) reduced cholesteryl ester transfer protein activity associated with decreased TRL and increased HDL-C levels; and finally, (5) increased expression of apo A-I and apo A-II in liver contributing to elevated HDL-C levels [4]. Previous kinetic studies using fibrates in patients with hypertriglyceridemia or insulin resistance reported an increase of TRL apo B catabolism with no change in TRL apo B PR [36,38,51]. In the present study, the TG-lowering effect of fenofibrate resulted from enhanced catabolism of both apo B-48– and apo B-100–containing TRLs associated with elevated LPL activity. Furthermore, the IDL PR was increased by 58.2% after treatment with fenofibrate; and VLDL particles were more rapidly converted to IDL and then removed from the circulation. In fact, the increase in IDL apo B-100 catabolism after fenofibrate therapy prevented a significant increase in IDL PS secondary to enhanced conversion of VLDL to IDL. Very low-density lipoprotein apo B-100 production remained unchanged, thereby supporting the previous finding that fenofibrate has no effect on hepatic TRL secretion [36,38,51]. The lipid composition of VLDL remained unaltered by fenofibrate treatment because VLDL cholesterol and TG were reduced equally (−50% and −47%).

In summary, the results of this study showed that in patients with type 2 diabetes mellitus with marked hypertriglyceridemia, atorvastatin and fenofibrate decrease plasma TG levels to the same extent. Atorvastatin increases the catabolic rate of VLDL and IDL apo B-100, presumably by up-regulating LDL receptor–related protein or other receptors not specifically involved in LDL particle clearance, whereas fenofibrate appears to elevate TRL apo B-48 as well as VLDL and IDL apo B-100 catabolic rates by increasing intravascular lipolytic efficiency. Atorvastatin reduces TRL apo B-48 PR, presumably by decreasing intestinal cholesterol absorption and chylomicron synthesis. Finally, the decrease in plasma LDL-C and apo B levels after treatment with atorvastatin in these diabetic patients was attributed to reduced rate of LDL production.

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