

Effects of atorvastatin versus fenofibrate on apoB-100 and apoA-I kinetics in mixed hyperlipidemia

Stefan Bilz,* Stephan Wagner,[†] Michaela Schmitz,[†] Andrea Bedynek,[†] Ulrich Keller,^{1,*} and Thomas Demant^{†,§}

Division of Endocrinology, Diabetology, and Clinical Nutrition,* University Hospital Basel, Basel, Switzerland; Institute of Clinical Chemistry,[†] Klinikum der Ludwig-Maximilians-University, Grosshadern, Munich, Germany; and Institute of Clinical Chemistry,[§] Krankenhaus Dresden-Friedrichstadt, Dresden, Germany

Abstract Kinetics of apo B and apo AI were assessed in 8 patients with mixed hyperlipidemia at baseline and after 8 weeks of atorvastatin 80 mg q.d. and micronised fenofibrate 200 mg q.d. in a cross-over study. Both increased hepatic production and decreased catabolism of VLDL accounted for elevated cholesterol and triglyceride concentrations at baseline. Atorvastatin significantly decreased triglyceride, total, VLDL and LDL cholesterol and apo B concentrations (−65%, −36%, −57%, −40% and −33%, respectively, $P < 0.05$). Kinetic analysis revealed that atorvastatin stimulated the catabolism of apo B containing lipoproteins, enhanced the delipidation of VLDL₁ and decreased VLDL₁ production. Fenofibrate lowered triglycerides and VLDL cholesterol (−57% and −64%, respectively, $P < 0.05$) due to enhanced delipidation of VLDL₁ and VLDL₂ and increased VLDL₁ catabolism. Changes of HDL particle composition accounted for the increase of HDL cholesterol during atorvastatin and fenofibrate (18% and 23%, $P < 0.01$). Only fenofibrate increased apo AI concentrations through enhanced apo AI synthesis (45%, $P < 0.05$). We conclude that atorvastatin exerts additional beneficial effects on the metabolism of apo B containing lipoproteins unrelated to an increase in LDL receptor activity. Fenofibrate but not atorvastatin increases apo AI production and plasma turnover.—Bilz, S., S. Wagner, M. Schmitz, A. Bedynek, U. Keller, and T. Demant. Effects of atorvastatin versus fenofibrate on apolipoprotein B-100 and apolipoprotein A-I kinetics in mixed hyperlipidemia. *J. Lipid Res.* 2004. 45: 174–185.

Supplementary key words apolipoprotein metabolism • stable isotopes • multicompartmental model

Mixed hyperlipidemia, i.e., the increase of both plasma total cholesterol and triglycerides, refers to an etiologically heterogeneous lipoprotein phenotype that occurs in both primary and secondary dyslipidemias. The usually associated decrease of HDL cholesterol and the shift in the LDL size profile toward small, dense particles further con-

tribute to the considerable cardiovascular risk in affected patients (1).

Among the currently available lipid-lowering compounds, HMG-CoA reductase inhibitors, also referred to as statins, and fibrates have been proven to effectively decrease cardiovascular morbidity and mortality in several large-scale primary and secondary prevention trials during the past two decades (2–8). Thus, current guidelines recommend the use of these drugs both in patients with established cardiovascular disease and in subjects at risk as a result of preexisting hyperlipidemia (9). In patients with mixed hyperlipidemia and moderately to severely increased plasma triglycerides, therapy with either compound may result in favorable changes of the lipoprotein pattern. Classic studies demonstrated that the dose-dependent decreases in plasma total and LDL cholesterol levels during competitive inhibition of HMG-CoA reductase by statin therapy are secondary to enhanced LDL receptor-mediated lipoprotein catabolism (10). More recently, the triglyceride-lowering capacity of statins has gained additional attention, and atorvastatin has been found to be particularly efficacious, most likely secondary to its longer duration of action and its enhanced lipid-lowering potency (11, 12). Enhanced catabolism of apolipoprotein B (apoB)-containing triglyceride-rich lipoproteins via the LDL receptor pathway may in part explain the decrease in plasma triglycerides elicited by statins. However, data obtained from patients with LDL receptor-negative homozygous familial hypercholesterolemia and animal models clearly suggest that statins may also interfere with hepatic lipoprotein production (13, 14). The effects of fibrates, among them fenofibrate, on plasma lipids have been found to result from the activation of the peroxisome proliferator-activated receptor α (PPAR α) (15). Specifically,

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; d₃-leucine, deuterated leucine; FCR, fractional catabolic rate; LPL, lipoprotein lipase; PPAR α , peroxisome proliferator-activated receptor α .

¹ To whom correspondence should be addressed.

e-mail: ukeller@uhbs.ch

Manuscript received 11 July 2003 and in revised form 17 September 2003.

Published, JLR Papers in Press, October 1, 2003.

DOI 10.1194/jlr.M300309JLR200

fibrates have been demonstrated to reduce hepatic triglyceride production, to enhance delipidation and clearance of triglyceride-rich lipoproteins, and to activate reverse cholesterol transport (16). These alterations in lipid metabolism are associated with a profound decrease of plasma triglycerides, increased HDL cholesterol levels, and a favorable shift in the density distribution of LDL toward buoyant, less-atherogenic particles, whereas the effects on LDL cholesterol levels may vary. However, to our knowledge, data regarding the comparative effects of statins and fibrates on lipoprotein metabolism in the subset of patients with mixed hyperlipidemia and moderately to severely increased plasma triglyceride levels are not available from the literature. Therefore, we performed apoB and apolipoprotein A-I (apoA-I) kinetic studies using endogenous labeling of apoB and apoA-I with deuterated leucine (d_3 -leucine) and multicompartmental modeling to derive lipoprotein production and catabolic rates in the basal state and after 8 weeks of therapy with either atorvastatin or fenofibrate.

METHODS

Subjects

Eight male patients (age 51 ± 4 years, mean body weight 86 ± 4 kg) with increased fasting triglyceride (3.7 – 26 mmol/l) and normal to increased LDL cholesterol (2.4 – 5.9 mmol/l) levels were selected from the lipid outpatient clinic at Basel University Hospital. Eight male subjects with normal fasting plasma triglyceride levels matched for age (48 ± 3 years) and weight (86 ± 6 kg) served as controls. The data for the control subjects were taken from a previous publication (17). ApoA-I kinetic data for hyperlipidemic patients at baseline were compared with data from a group of healthy controls (age 25 ± 0.4 years, mean body weight 73 ± 2 kg). Plasma lipids and lipoproteins were analyzed according to standard procedures (18). None of the study participants had diabetes mellitus, cholestasis, nephrotic syndrome, pancreatitis, primary hypothyroidism, renal or hepatic dysfunction, or chronic alcoholism. One patient (patient 7) suffering from panhypopituitarism was on stable replacement doses of levothyroxine, cortisol, recombinant human growth hormone, and testosterone throughout the study. Immunosuppressive agents, imidazole antimycotics, macrolide antibiotics, β blocking agents, diuretics, and isotretinoin were not allowed during the study period, nor were lipid-lowering agents other than the study medication.

Study design

The study used an open, randomized, crossover design that consisted of two treatment periods of 8 weeks each. After discontinuing all preexisting lipid-lowering drugs, patients entered a 6 week run-in period and underwent the baseline apoB turnover study during the last 2 weeks of this period. Thereafter, they were randomized to receive either atorvastatin (80 mg/d) or micronized fenofibrate (200 mg/d) for 8 weeks together with their evening meal. After a washout period of 4 weeks, patients were switched to the other study drug. ApoB turnover studies were performed during the last 2 weeks of both treatment phases.

All patients received dietary counseling by a dietitian immediately after inclusion into the study protocol and two times thereafter, once during each treatment period. They were asked to follow an isoenergetic diet containing 50% to 55% carbohydrates,

15% to 20% proteins, and 30% fats, with less than 300 mg of cholesterol and less than 10% of total energy as saturated fatty acids. Alcohol consumption of one drink or less per day was allowed, and patients were asked to refrain from vigorous physical exercise during the study period. Compliance with the dietary regimen was assessed by detailed food protocols of 3 days, including 1 weekend day, during each study period.

Study medication

The Clinical Pharmaceutical Operations Department of Warner Lambert provided 40 mg atorvastatin tablets. Micronized fenofibrate capsules (200 mg) were purchased from Fournier Pharmaceutical Company (Schwarz Pharma AG, Switzerland). Patients were instructed to take two atorvastatin 40 mg tablets or one micronized fenofibrate 200 mg capsule together with their evening meal during the treatment periods. Compliance with the study medication was assessed by pill counting. The lipid-lowering drugs were well tolerated in all but one patient, who developed a macular rash during fenofibrate therapy and therefore discontinued the drug.

Turnover protocol

The detailed procedures for conducting the turnover study have been reported previously (19). In brief, subjects fasted for 11 h overnight before starting the study at 7 AM in a metabolic ward. After insertion of intravenous lines for tracer administration and sample collection, controls received either an intravenous bolus injection of d_3 -leucine (6.0 mg/kg body weight) or a primed constant infusion (0.6 mg/kg, followed by 0.6 mg/kg per h for 10 h). Patients received a modified primed constant infusion (1.2 mg/kg, followed by 1.2 mg/kg per h for 5 h), which allowed for a better definition of VLDL₁ tracer enrichment. The total dosage of tracer was nearly identical in all studies, and the three dosage schemes had been compared in previous studies, including the use of dual tracer applications (19, 20). In the first 10 h period, patients and controls continued to fast but were allowed noncaloric drinks and remained ambulatory in the metabolic ward. At 6 PM, all study participants were allowed to leave the hospital. Blood samples were collected in EDTA-containing tubes before administration of tracer and thereafter at 0.16, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 15, and 24 h in controls and at 0.25, 0.5, 1, 2, 3, 4, 5, 5.33, 5.66, 6, 7, 10, and 24 h in patients. The additional samples taken in patients at 5, 5.33, and 5.66 h were necessary because of the rapid decline of plasma d_3 -leucine concentrations after stopping the tracer infusion. Further fasting samples were obtained at approximately 8 AM daily for the next 10–14 days.

Lipoprotein isolation and preparation of apoB

The preparation of VLDL subfractions VLDL₁ [Svedberg flotation unit (S_f) 60–400] and VLDL₂ (S_f 20–60), intermediate density lipoprotein (IDL; S_f 12–20), LDL (S_f 0–12), LDL₁ (S_f 6–12), and LDL₂ (S_f 0–6) has been described previously and is based on the procedure of Lindgren, Jensen, and Hatch (19, 21). Briefly, 2 ml of plasma was adjusted to a density (d) of 1.118 g/ml by the addition of 0.03410 g of solid NaCl and layered over a 0.5 ml cushion of $d = 1.182$ g/ml NaBr solution in a Beckman SW 40 rotor tube. A discontinuous six-step salt gradient was constructed above this, and VLDL₁, VLDL₂, and IDL were harvested as described (19). Finally, LDL₁ and LDL₂ were isolated after further centrifugation at 36,000 rpm for 5 h, 6 min and at 32,000 rpm for 12 h, 9 min, respectively. Total LDL was reconstituted by mixing equal volumes of LDL₁ and LDL₂. From the infranatant, the HDL fraction was prepared after adjusting density to 1.21 g/ml by centrifugation at 40,000 rpm for 16 h in a Beckman 50.4 Ti

rotor. From each apoB-containing lipoprotein fraction, apoB was precipitated by the addition of an equal volume of isopropanol at room temperature (22). The pellet was delipidated with ethanol-ether (3:1) and dried with ether until apoB remained as a fine white protein pellet. HDL apoA-I was prepared by SDS-PAGE using an apoA-I standard, and apoA-I-containing bands were cut from the gel.

Preparation and analysis of leucine in apoB, apoA-I, and plasma

ApoB and apoA-I were hydrolyzed in glass tubes (Schott, Mainz, Germany) in the presence of 0.5–1.0 ml of 6 N HCl at 110°C for 20–24 h. The amino acid hydrolysate was concentrated in a vacuum concentrator centrifuge (Univapo 150 H; Uniequip, Martinsried/Munich, Germany) and aliquoted into microvials (Chromacol, Herts, UK). After complete removal of HCl, samples were ready for derivatization and mass spectrometric analysis.

Proteins were precipitated from 1 ml of plasma by adding 1 ml of TCA (10%), and amino acids were prepared from the supernatant by cation-exchange chromatography using 2 ml columns filled with Dowex AG-50W-X8 resin (H⁺-form, 50–100 mesh; Bio-Rad, Richmond, CA). The amino acids that bound to the resin were desorbed by 4 M NH₄OH, which was subsequently removed by evaporation in a vacuum pump (Univapo 150 H), transferred into microvials, and dried again for derivatization.

The method used for the analysis of d₃-leucine enrichment in protein hydrolysates and plasma amino acids is presented in detail elsewhere (19). Ion mass fragments at *m/z* 277, 276, and 274 were monitored by selective ion recording. The *m/z* 277:276 ratio showed a linear relationship with isotopic enrichment over the range 0.0–10.0% atom percent excess. The ratio of *m/z* 277:276 was multiplied by an average value for the constant ratio of *m/z* 276:274 (determined repeatedly throughout the analytical run, it shows no change over the range 0.0–10% atom percent excess), and the resulting *m/z* 277:274 values were used to calculate specific isotopic enrichments and tracer-tracee ratios (19).

Measurement of apoB and apoA-I masses in lipoprotein fractions

The apoB content of VLDL₁, VLDL₂, IDL, and LDL was determined as the difference between total and isopropanol-soluble protein measured by the procedure of Lowry et al. (23). Because more than 90% of plasma apoA-I is associated with HDL, the plasma apoA-I concentration was considered to equal the HDL apoA-I concentration. From apoB and apoA-I concentrations (in milligrams per milliliter of plasma) and an estimate of the plasma volume (4% of the body weight), apoB and apoA-I plasma pools were determined for the lipoprotein fractions of interest. The leucine content of the apoB and apoA-I pools was then calculated assuming leucine contents of 12.12% and 15.68%, respectively.

Kinetic analyses and multicompartmental modeling

The change in tracer-tracee ratios with time in plasma and in apoB in the four apoB-containing lipoprotein fractions, together with the measured apoB pool size for VLDL₁, VLDL₂, IDL, and LDL, was used as the data set for the derivation of apoB kinetic parameters. **Figure 1A** shows the multicompartmental model of apoB metabolism, which was constructed using the SAAM II modeling program (SAAM Institute, Seattle, WA), the development of which has been described (19). Briefly, its basic features include a four-compartment representation of free leucine kinetics (compartments 1–4), a sequence of lipoprotein compartments accounting for the stepwise delipidation of VLDL₁ through VLDL₂ and IDL to LDL (compartments 6, 7, 9, 10, 12,

and 14), plus the three remnant compartments 8, 11, and 13 for VLDL₁, VLDL₂, and IDL particles that are removed directly from plasma. Free leucine and the apoB-containing compartments 6, 9, 12, and 14 in the VLDL₁, VLDL₂, IDL, and LDL density range are linked via delay compartment 5, which is set at 0.5 h, the time required for apoB biosynthesis. Compartment 15 allows for some intravascular/extravascular exchange of LDL, which is not observed for less-dense lipoproteins. Typical examples of the time courses of leucine tracer-tracee ratios measured in the four apoB-containing lipoproteins VLDL₁, VLDL₂, IDL, and LDL at baseline and during therapy with each of the study drugs are shown in **Fig. 2A**. Similarly, apoA-I kinetic data were derived from changes in the tracer-tracee ratios in HDL apoA-I over time and in the apoA-I pool size in this lipoprotein fraction. **Figure 2B** shows a typical example of the time course of the leucine tracer-tracee ratio in HDL-associated apoA-I. The model to describe HDL apoA-I metabolism is shown in **Fig. 1B** and has been published previously (24). As for the apoB model, compartments 1–4 describe plasma leucine metabolism. An intrahepatic delay compartment accounts for hepatic synthesis and the secretion of apoA-I and is connected to a single intravascular compartment (compartment 6), from which apoA-I is cleared. The exchange of apoA-I with an extracellular pool is accounted for by the addition of compartment 7 to the model.

Ethical considerations

All subjects participating in this study gave written informed consent. The study was approved by the Ethics Committee of Basel University Hospital.

Statistical analyses

All statistical analyses were performed using Statistica 6 software (StatSoft, Inc., Tulsa, OK). Values are given as means ± SEM. Plasma lipids and lipoproteins during different treatments were analyzed using Student's *t*-test. The effects of treatments on lipoprotein kinetics data were assessed using the Wilcoxon test. Pearson correlation coefficients were used to express the relation between kinetic variables. Comparisons between patients at baseline and controls were performed using the Kruskal-Wallis test.

RESULTS

Lipids and apoB and apoA-I kinetic data of patients and controls at baseline

Table 1 demonstrates that both plasma total cholesterol and triglycerides were increased markedly in patients (7.23 ± 1.39 and 8.84 ± 7.38 mmol/l, respectively). Whereas the increase in total cholesterol was secondary to the increased VLDL cholesterol levels in most patients, some presented also with increased LDL cholesterol. Compared with patients, age- and sex-matched controls showed significantly lower plasma triglyceride concentrations and VLDL cholesterol levels. Total and LDL cholesterol levels, however, did not differ significantly. HDL cholesterol levels were decreased compared with those of both age- and sex-matched and nonmatched normolipidemic controls.

Table 2 demonstrates that in patients, there was a 2-fold increase in apoB secretion into the VLDL₁ density range compared with controls. This was associated with decreased rates of transfer of VLDL₁ to VLDL₂ and a diminished fractional catabolic rate (FCR) of VLDL₁. A sig-

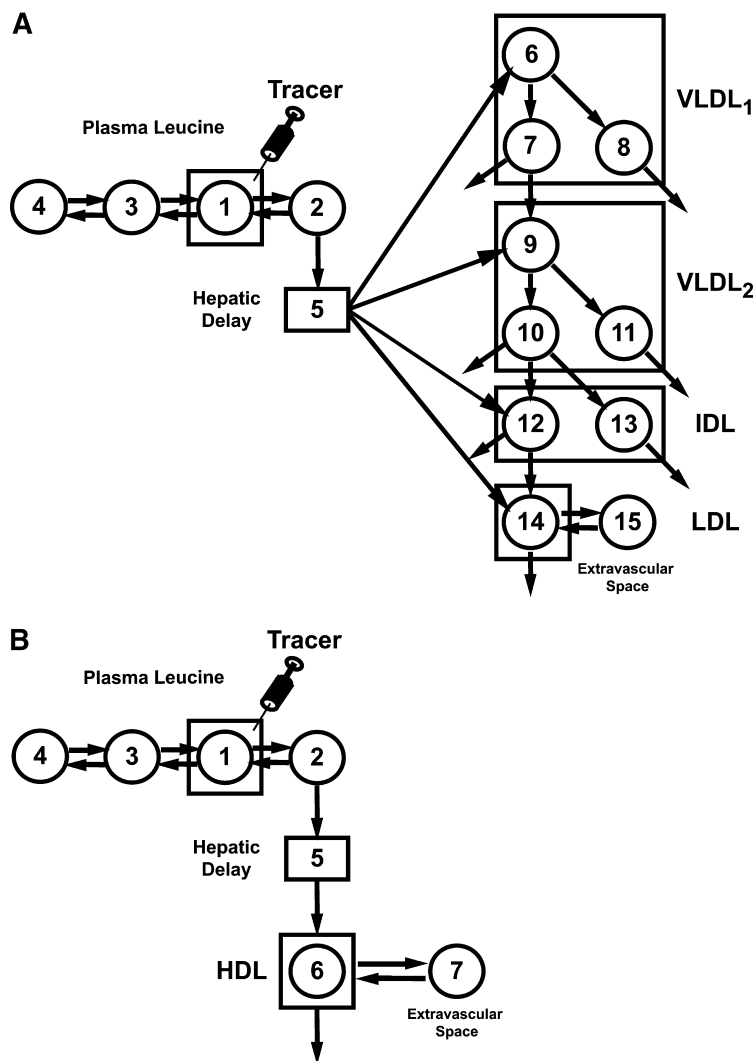


Fig. 1. A: Multicompartmental model of apolipoprotein B (apoB) metabolism. Because plasma-free tracer kinetics are fully accounted for, both bolus and primed constant infusion data could be analyzed using the same model. Plasma leucine is represented by a compartment (compartment 1) that received the tracer and distributed it to body protein pools (compartments 3 and 4) and an intracellular compartment (compartment 2) that was the precursor to apoB synthesis. Compartment 5 represents an intrahepatic pool accounting for the delay (0.5 h) associated with the synthesis of apoB, lipoprotein assembly, and secretion. Compartments 6–9, 12, and 14 form a delipidation chain, and tracer appeared throughout this chain, i.e., in VLDL₁ (compartment 6), VLDL₂ (compartment 9), intermediate density lipoprotein (IDL; compartment 12), and LDL (compartment 14). Compartments 8, 11, and 13 represent lipoprotein remnants that are directly removed from the circulation. Compartment 15 accounts for an extravascular exchange of LDL, which does not occur for the other apoB-containing lipoproteins. A detailed description of the model is given in ref. (19). B: Multicompartmental model of apolipoprotein A-I (apoA-I) metabolism. Plasma leucine is represented by a compartment (compartment 1) that received the tracer and distributed it to body protein pools (compartments 3 and 4) and an intracellular pool (compartment 2). Compartment 5 represents an intrahepatic pool accounting for the delay (0.15 h) associated with the synthesis of apoA-I, lipoprotein assembly, and secretion. All tracer input into HDL apoA-I occurs in a single compartment (compartment 6) from which apoA-I is cleared. Compartment 7 accounts for an extravascular HDL pool.

nificant negative correlation between VLDL₁ apoB concentrations and VLDL₁ apoB transfer rates was observed at baseline ($r = -0.72$, $P = 0.04$). Although the conversion rate of VLDL₁ to VLDL₂ was delayed in patients, the marked increase in the VLDL₁ apoB pool resulted in an increase in the absolute amount of apoB transferred from the VLDL₁ to the VLDL₂ compartment. The consecutive increase in VLDL₂ apoB pool size in patients was further enhanced by a lower rate of apoB transfer from VLDL₂ to IDL, which was only in part compensated for by the enhanced direct catabolism of VLDL₂. Direct production of VLDL₂ apoB was not different between the groups. Despite a slight but statistically significant increase in IDL apoB production from VLDL₂ in patients, the IDL apoB pool size was similar in patients and controls (Tables 3, 4; Fig. 3). Finally, LDL apoB kinetic parameters did not differ significantly, although there was a trend toward higher LDL apoB FCR in patients (Table 5, Fig. 3). Furthermore, both LDL cholesterol and LDL apoB concentrations were inversely correlated with the rate of LDL catabolism in our patients at baseline ($r = -0.85$, $P = 0.002$ and $r = -0.89$, $P = 0.006$, respectively). The lipid composition of VLDL₁ and VLDL₂ particles in patients

and controls was comparable (data not shown). Thus, the observed differences in triglyceride and VLDL cholesterol concentrations in our patients were accounted for by hepatic oversecretion, impaired direct catabolism, and delayed delipidation of normally composed VLDL₁. This led to an increase in the number of VLDL₂ particles, which were also metabolized more slowly, further contributing to the observed hypertriglyceridemia and hypercholesterolemia.

The decrease in plasma HDL cholesterol concentrations was in part accounted for by compositional changes of HDL particles, which were triglyceride enriched (9.6 ± 1.2 vs. $3.5 \pm 0.4\%$; $P < 0.001$) and cholesterol depleted (11.6 ± 0.6 vs. $17.6 \pm 0.6\%$; $P < 0.001$) compared with the group of non age- and sex-matched controls. The lower apoA-I plasma concentrations in hypertriglyceridemic subjects (1.13 ± 0.07 vs. 1.33 ± 0.10 ; $P = 0.046$) were associated with a trend toward an increase in apoA-I catabolism [0.21 ± 0.02 vs. 0.28 ± 0.02 ; $P = 0.093$ (Table 6)].

Effects of atorvastatin and fenofibrate on plasma lipids, apolipoproteins, and apoB and apoA-I kinetics

Therapy with atorvastatin (80 mg/d) decreased total, VLDL, and LDL cholesterol by 39%, 52%, and 40%, re-

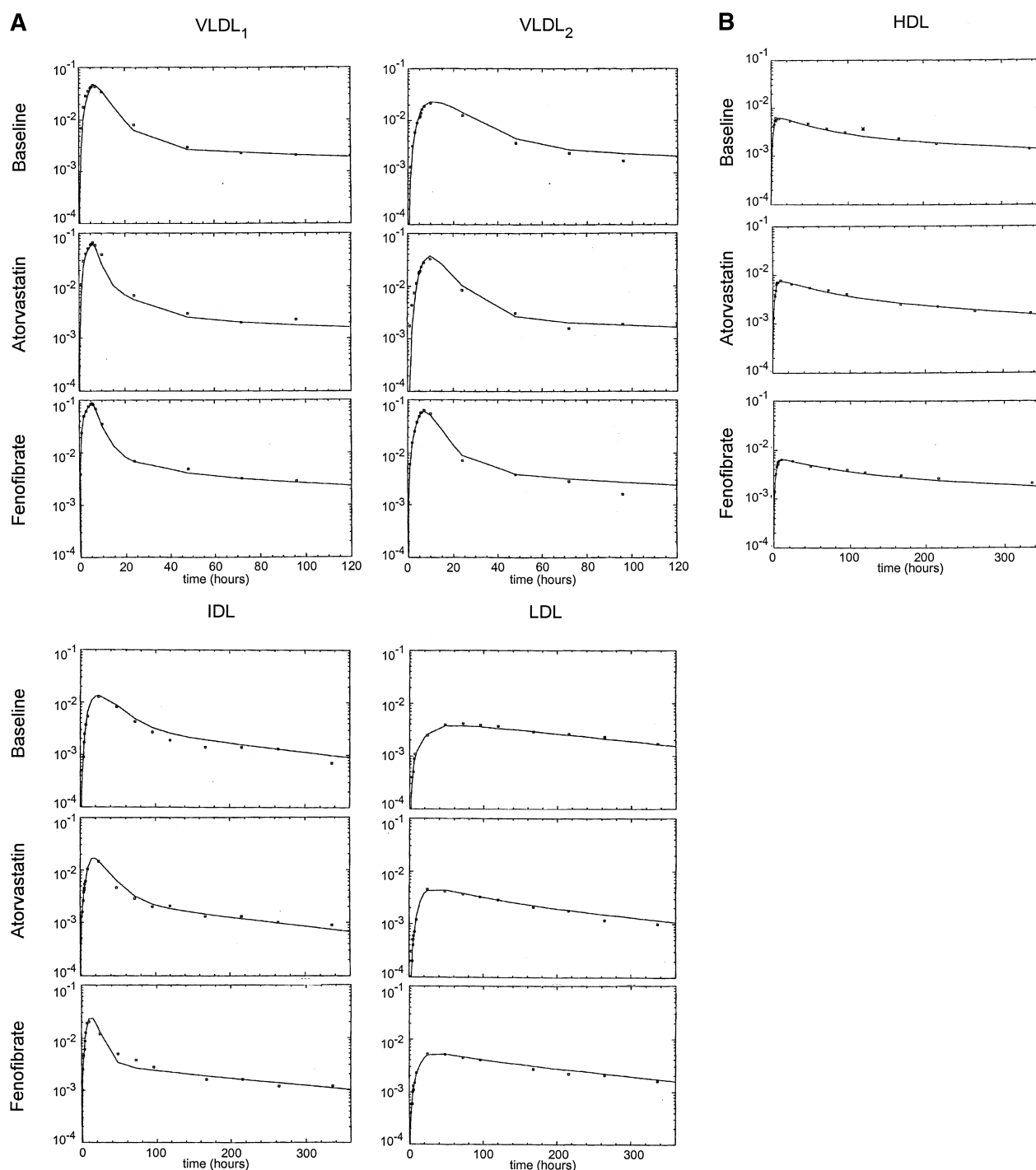


Fig. 2. Tracer-tracee ratios recorded after a primed constant infusion of deuterated leucine as a metabolic tracer. ApoB from VLDL₁, VLDL₂, IDL, and LDL (A) and apoA-I from HDL (B) at baseline and during atorvastatin and fenofibrate treatment are shown from patient 7.

spectively ($P < 0.02$). Fasting triglycerides decreased by 57%, and a statistically significant 18% increase in HDL cholesterol was observed ($P < 0.02$). Whereas plasma triglycerides and VLDL cholesterol were decreased equally during fenofibrate therapy (−57% and −54%; $P < 0.02$), the decrease of plasma total cholesterol was significantly less-pronounced compared with that during atorvastatin therapy (−17%; $P < 0.03$ and 0.02 vs. baseline and atorvastatin, respectively). With fenofibrate therapy, LDL chole-

sterol and apoB plasma concentrations remained unchanged. Furthermore, the increase in the ratio of LDL₁ to LDL₂ during fenofibrate therapy indicated a shift of LDL particle size toward larger, buoyant LDLs.

ApoB kinetic data revealed a marked decrease in VLDL₁ apoB pool size during treatment with atorvastatin (−62%; $P = 0.012$), attributable to an increased fractional transfer rate of VLDL₁ apoB to VLDL₂ (+140%; $P = 0.012$). Furthermore, direct hepatic VLDL₁ apoB production de-

TABLE 1. Lipid and lipoprotein values in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Cholesterol	Triglycerides	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol	apoB	apoA-I	LDL ₁ /LDL ₂
	mmol/l			g/l				
Controls								
C1	5.64	1.16	0.49	3.67	1.50			
C2	5.53	0.76	0.34	3.98	1.19			
C3	6.34	1.39	0.57	4.50	1.29			
C4	6.93	1.60	0.75	5.30	0.93			
C5	6.26	1.94	0.80	4.16	1.32			
C6	6.93	1.55	0.85	4.99	1.09			
C7	6.21	2.10	1.24	4.09	0.85			
C8	6.23	2.39	0.96	3.54	0.96			
Mean	6.26	1.61	0.75	4.28	1.14			
SEM	0.18	0.19	0.10	0.22	0.08			
Patients								
Baseline								
P1	9.90	26.13	7.03	2.38	0.49	1.11	0.87	0.84
P2	7.16	11.36	2.87	3.41	0.88	1.44	1.22	0.40
P3	6.47	7.51	3.34	2.38	0.78	1.25	1.04	0.76
P4	6.21	7.11	2.59	2.87	0.70	1.22	0.99	0.36
P5	6.26	5.37	2.64	2.72	0.88	1.05	1.39	0.37
P6	6.98	4.98	2.09	4.06	0.83	1.47	1.17	1.06
P7	8.79	4.56	2.12	5.92	0.67	1.95	1.03	0.45
P8	6.10	3.74	1.66	3.62	0.83	1.40	0.97	0.63
Mean	7.23	8.84 ^a	3.04 ^b	3.42	0.76 ^b	1.36	1.09	0.61
SEM	0.49	2.61	0.60	0.42	0.05	0.10	0.06	0.09
Atorvastatin								
P1	3.90	4.65	2.15	1.19	0.57	0.80	0.87	0.69
P2	5.07	3.32	1.40	2.56	1.11	1.05	1.26	0.73
P3	3.78	3.94	1.47	1.29	1.03	0.74	1.21	0.32
P4	3.85	3.33	1.45	1.73	0.70	0.88	0.94	0.30
P5	3.90	2.61	1.16	1.66	1.11	0.73	1.43	0.52
P6	4.47	3.00	1.34	2.17	0.96	0.91	1.22	0.51
P7	4.73	2.51	1.14	2.79	0.80	1.10	1.04	0.70
P8	4.50	1.40	0.65	2.95	0.91	1.08	1.03	0.55
Mean	4.28 ^{c,d}	3.09 ^e	1.34 ^c	2.04 ^{c,d}	0.90 ^c	0.91 ^{c,d}	1.13	0.54 ^d
SEM	0.17	0.35	0.15	0.24	0.07	0.05	0.07	0.06
Fenofibrate								
P1	5.66	6.88	2.66	2.40	0.59	1.12	1.03	1.90
P2	6.65	3.59	1.32	4.32	1.03	1.50	1.30	0.78
P3	5.20	2.34	1.06	3.18	0.96	1.21	1.11	1.19
P5	5.07	2.13	0.93	2.87	1.27	1.00	1.57	1.50
P6	5.53	2.53	1.03	3.41	1.09	1.18	1.35	1.97
P7	7.03	2.17	0.91	5.33	0.80	1.65	1.02	1.19
P8	6.44	2.71	1.29	4.42	0.83	1.53	1.04	0.86
Mean	5.94 ^e	3.19 ^e	1.32 ^c	3.71	0.94 ^c	1.31	1.20 ^c	1.34 ^c
SEM	0.29	0.64	0.23	0.39	0.08	0.09	0.08	0.18

apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; C1–C8, controls 1–8; P1–P8, patients 1–8.

^a $P < 0.05$ versus controls.^b $P < 0.01$ versus controls.^c $P < 0.01$ versus baseline.^d $P < 0.01$ versus fenofibrate.^e $P < 0.05$ versus baseline.

creased in six of eight patients. Remarkably, the patients showing no decrease of VLDL₁ production when treated with atorvastatin (patients 5 and 8) had the lowest VLDL₁ production rates at baseline and experienced a pronounced increase in VLDL₁ direct catabolism (Table 2, Fig. 3).

Because direct hepatic VLDL₂ production and input of apoB from VLDL₁ did not change, the lower VLDL₂ apoB pool in atorvastatin-treated subjects (–43%; $P = 0.012$) was attributable to enhanced direct removal of VLDL₂ particles from the circulation (+67%; $P = 0.035$). IDL and LDL apoB pools were reduced by atorvastatin therapy (–48% and –33%, respectively; $P = 0.012$). Direct hepatic production rates of these lipoproteins were not al-

tered, but there was a trend toward a faster elimination of IDL particles. The reduction of LDL apoB pool size with atorvastatin therapy resulted from both decreased LDL apoB production and increased LDL catabolism. LDL production, defined as the sum of direct secretion and input from IDL, was reduced significantly (–24%; $P = 0.05$), whereas LDL FCR was increased in seven of eight patients.

The striking effect of fenofibrate on VLDL₁ apoB pool size resulted from both enhanced direct catabolism (+239%; $P = 0.043$) and increased fractional transfer to VLDL₂ (+147%; $P = 0.018$). Hepatic VLDL₁ apoB secretion was not significantly affected. Additionally, a marked acceleration of the delipidation of VLDL₂ was observed

TABLE 2. VLDL₁ apoB kinetics in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Production	Pool	Transfer	FCR
	mg/kg/day	mg	pools/day	
Controls				
C1	12.05	59	9.90	7.20
C2	7.37	27	10.40 ^d	9.80
C3	26.59	123	5.70	11.10
C4	12.10	127	5.90	4.00
C5	11.47	136	3.20	1.70
C6	6.67	78	7.60	0.10
C7	13.95	150	4.00	6.30
C8	15.47	193	3.90	3.20
Mean	13.21	112	6.33	5.43
SEM	2.18	19	0.97	1.37
Patients				
Baseline				
P1	22.11	1,515	0.49	0.72
P2	47.72	1,023	1.27	2.69
P3	29.13	1,102	1.49	1.13
P4	26.36	847	1.98	1.35
P5	14.66	610	1.04	0.68
P6	29.06	673	2.49	1.48
P7	20.10	453	1.27	2.02
P8	19.56	430	2.48	1.03
Mean	26.09 ^a	832 ^a	1.56 ^a	1.39 ^a
SEM	3.56	131	0.25	0.24
Atorvastatin				
P1	15.11	479	2.40	0.22
P2	17.78	355	1.82	2.43
P3	9.90	341	2.83	0.05
P4	17.12	425	2.25	2.06
P5	25.13	310	2.37	3.46
P6	20.37	296	6.15	0.18
P7	15.59	190	4.21	1.87
P8	22.80	160	7.97	3.00
Mean	17.98	320 ^b	3.75 ^b	1.66 ^c
SEM	1.69	38	0.78	0.48
Fenofibrate				
P1	29.77	614	2.56	1.46
P2	43.40	371	3.49	6.44
P3	20.04	190	3.11	7.34
P5	28.40	149	4.67	9.10
P6	17.75	215	4.09	3.52
P7	21.89	198	3.24	4.93
P8	16.05	207	5.83	0.15
Mean	25.33	278 ^d	3.86 ^d	4.71 ^d
SEM	3.58	62	0.42	1.22

FCR, fractional catabolic rate.

^a $P < 0.01$ versus controls.

^b $P < 0.01$ versus baseline.

^c $P < 0.05$ versus fenofibrate (Wilcoxon test).

^d $P < 0.05$ versus baseline.

TABLE 3. VLDL₂ apoB kinetics in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Production	Flux	Pool	Transfer	FCR
	mg/kg/day		mg	pools/day	
Controls					
C1	3.37	6.98	114	7.30	0.20
C2	3.61	3.79	96	4.20	0.50
C3	4.67	9.01	182	5.00	0.80
C4	2.21	7.22	236	2.20	0.10
C5	1.55	7.50	156	3.20	0.20
C6	6.20	6.56	267	1.80	0.00
C7	4.17	5.47	462	2.20	0.00
C8	3.98	8.46	321	3.00	0.40
Mean	3.72	6.87	229	3.61	0.28
SEM	0.51	0.59	43	0.65	0.10
Patients					
Baseline					
P1	12.24	9.02	1,656 ^a	0.38	0.69
P2	6.29	15.31	487	1.39	2.38
P3	2.14	16.57	513	2.05	1.56
P4	4.48	15.70	654	1.97	1.34
P5	2.96	8.86	365	2.30	0.03
P6	1.99	18.25	438	2.67	1.58
P7	5.13	7.76	500	1.91	0.00
P8	4.17	13.84	447	2.20	0.91
Mean	4.93	13.16 ^a	633 ^a	1.86 ^b	1.06 ^b
SEM	1.17	1.43	149	0.25	0.29
Atorvastatin					
P1	5.28	13.84	528	1.56	1.44
P2	9.47	7.62	354	1.69	2.41
P3	3.44	9.74	512	1.23	1.32
P4	10.20	8.94	517	1.96	2.00
P5	2.42	10.21	189	1.95	2.86
P6	3.42	19.79	281	5.79	1.81
P7	0.28	10.80	318	2.23	0.35
P8	0.46	16.57	181	5.26	1.98
Mean	4.37	12.19	360 ^c	2.71	1.77 ^{d,e}
SEM	1.33	1.48	51	0.63	0.27
Fenofibrate					
P1	3.81	18.95	760	1.58	0.90
P2	2.78	15.27	349	4.32	0.08
P3	2.72	5.97	273	2.97	0.18
P5	0.00	9.64	148	3.50	1.20
P6	4.73	9.54	281	3.69	0.98
P7	4.62	8.69	208	3.73	1.00
P8	2.35	15.65	388	2.73	0.84
Mean	3.00	11.96	344 ^d	3.22 ^d	0.74
SEM	0.61	1.77	76	0.34	0.16

^a $P < 0.01$ versus controls.

^b $P < 0.05$ versus controls (Kruskal-Wallis test).

^c $P < 0.01$ versus baseline.

^d $P < 0.05$ versus baseline.

^e $P < 0.05$ versus fenofibrate (Wilcoxon test).

(+79%; $P = 0.018$), whereas no consistent effect on the direct catabolism of VLDL₂ could be demonstrated. The increase in fractional catabolism of IDL (+168%; $P = 0.028$) during fenofibrate therapy prevented an increase in IDL apoB pool size secondary to enhanced transfer from VLDL₂ to IDL. In contrast to the effects of fenofibrate on IDL precursors in the delipidation chain, the transformation of IDL to LDL was not stimulated. LDL apoB catabolism was decreased in all but one patient during fenofibrate treatment, leading to a statistically significant decrease (−17%; $P = 0.018$). ApoB pool size was significantly lower after therapy with atorvastatin compared with fenofibrate.

Although atorvastatin and fenofibrate increased HDL cholesterol levels to a similar degree (18% and 22%; $P =$

0.017 and 0.027, respectively), apoA-I plasma concentrations were only increased by fenofibrate. In accordance with this finding, no effects of atorvastatin on apoA-I kinetic parameters were found, whereas fenofibrate therapy increased both apoA-I production and, to a lesser extent, apoA-I catabolism (+37% and +21%, respectively; $P < 0.05$). Both atorvastatin and fenofibrate similarly reduced the triglyceride content of HDL particles (−34% and −38%, respectively; $P < 0.05$).

DISCUSSION

The present study provides a new additional explanation for the distinct triglyceride-lowering effect of the

TABLE 4. Intermediate density lipoprotein apoB kinetics in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Production	Flux	Pool	Transfer	FCR
	mg/kg/day		mg	pools/day	
Controls					
C1	1.57	10.08	433	1.69	0.55
C2	2.87	5.61	321	1.92	0.32
C3	1.86	11.65	488	1.85	0.31
C4	0.82	4.93	498	1.20	0.89
C5	0.24	8.66	370	1.14	0.25
C6	0.01	5.19	527	0.89	1.29
C7	3.87	9.20	663	2.19	0.07
C8	2.99	10.89	505	1.60	0.85
Mean	1.78	8.28	476	1.56	0.57
SEM	0.49	0.95	37	0.16	0.15
Patients					
Baseline					
P1	3.14	7.53	1,077	0.44	0.38
P2	3.87	7.96	330	3.05	0.00
P3	1.18	10.64	384	2.69	0.35
P4	0.27	12.02	502	1.71	0.91
P5	1.24	11.69	291	2.97	0.23
P6	0.46	12.71	782	1.55	0.00
P7	0.28	12.89	572	0.83	0.89
P8	0.59	12.75	438	1.60	0.75
Mean	1.38	11.02 ^a	547	1.86	0.44
SEM	0.49	0.76	94	0.34	0.13
Atorvastatin					
P1	1.78	9.94	280	1.13	2.34
P2	5.24	7.07	306	2.83	0.59
P3	2.53	6.36	204	2.54	1.77
P4	0.28	9.48	283	3.28	0.40
P5	1.68	5.13	167	2.88	0.06
P6	1.45	17.68	431	1.36	2.72
P7	1.26	9.58	285	1.78	1.03
P8	1.01	12.37	317	2.72	0.53
Mean	1.90	9.70	284 ^{b,c}	2.32	1.18
SEM	0.53	1.40	28	0.28	0.35
Fenofibrate					
P1	0.84	14.49	634	1.00	1.00
P2	6.53	17.73	351	3.00	2.88
P3	1.92	8.20	366	1.93	0.81
P5	2.74	7.19	247	2.20	0.69
P6	1.32	11.27	462	1.60	0.99
P7	1.62	10.48	435	1.34	0.72
P8	3.32	13.77	548	1.22	1.18
Mean	2.61	11.88	435	1.76	1.18 ^d
SEM	0.73	1.40	49	0.26	0.29

^a $P < 0.05$ versus controls (Kruskal-Wallis test).

^b $P < 0.01$ versus baseline.

^c $P < 0.05$ versus fenofibrate (Wilcoxon test).

^d $P < 0.05$ versus baseline.

HMG-CoA reductase inhibitor atorvastatin in subjects with mixed hyperlipidemia and contrasts its actions with those obtained during administration of fenofibrate, a fibrate derivative and PPAR α agonist.

The marked increase of triglyceride plasma concentrations in patients compared with controls was secondary to an increase in the number of circulating lipoprotein particles in the VLDL₁ and VLDL₂ density range, because the lipid composition of these lipoproteins remained unchanged. A marked increase in the hepatic production of VLDL₁, a pronounced delay in the delipidation of VLDL₁ to VLDL₂ and VLDL₂ to IDL, and a decrease in the direct catabolism of VLDL₁ accounted for the changes of plasma lipoprotein concentrations observed. These findings in

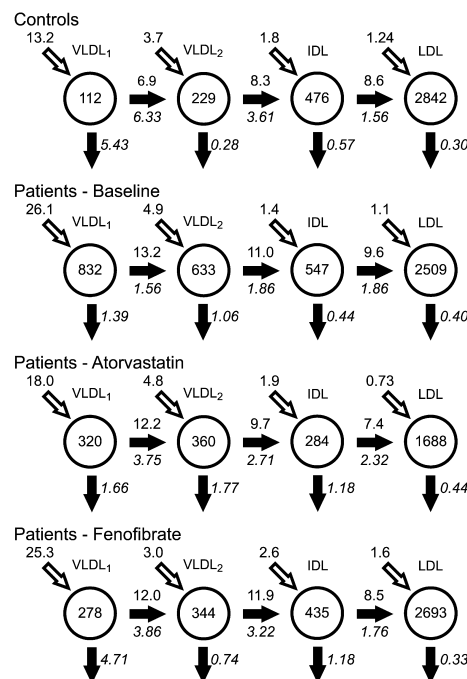


Fig. 3. Turnover of apoB in controls and patients at baseline and after 8 weeks of therapy with atorvastatin and fenofibrate. Mean apoB pool sizes (milligrams) in each lipoprotein fraction are shown in circles. Direct hepatic production and flux rates are given in milligrams per kilogram of body weight per day (open arrows). Fractional transfer rates and fractional rates of direct catabolism (pools per day, closed arrows) are shown in italic.

the basal state are in agreement with the results of several other apoB kinetic studies in patients with mixed hyperlipidemia showing both increased production of compositionally unaltered VLDL and delayed removal (25–28). The increased LDL cholesterol levels in some subjects were accounted for by lower LDL catabolic rates.

The decrease of HDL cholesterol and apoA-I concentrations in our hypertriglyceridemic subjects agrees with previously reported data (29). These changes were associated with an increase in HDL triglyceride content and accelerated apoA-I catabolism. In the presence of excess circulating triglyceride-rich lipoproteins, cholesterol ester transfer protein (CETP) mediates the reciprocal transfer of triglycerides from triglyceride-rich lipoproteins to HDL in exchange for cholesterol esters, thereby generating triglyceride-enriched HDL. Triglyceride-rich HDLs, however, are more susceptible to lipolytic modification by hepatic lipase, resulting in the formation of small, dense HDL particles, which are then more easily catabolized. This mechanism provides an explanation for the findings of our study (30, 31). Statins have consistently been shown to decrease plasma LDL cholesterol in response to an increase in hepatic LDL receptor expression and hence increased catabolism of apoB-containing lipoproteins (10). The increased catabolism of VLDL₂, IDL, and LDL apoB in most of our patients during atorvastatin treatment and the results of other recent apoB kinetic studies in subjects with mixed hyperlipidemia support this mechanism (32–

TABLE 5. LDL apoB kinetics in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Production	Flux	Pool	FCR
	mg/kg per day		mg	pools/day
Controls				
C1	0.01	8.81	2,612	0.28
C2	4.97	8.20	2,219	0.44
C3	3.47	11.55	2,791	0.42
C4	0.02	5.77	3,407	0.18
C5	0.47	7.29	1,901	0.24
C6	0.92	5.21	3,003	0.18
C7	0.02	13.07	3,853	0.38
C8	0.01	9.06	2,892	0.28
Mean	1.24	8.62	2,835	0.30
SEM	0.68	0.95	220	0.04
Patients				
Baseline				
P1	0.51	5.73	919	0.56
P2	1.93	11.83	2,933	0.40
P3	0.69	10.46	2,439	0.45
P4	1.14	8.02	2,371	0.41
P5	1.37	11.99	1,776	0.54
P6	0.40	13.17	3,653	0.34
P7	1.87	6.38	3,334	0.18
P8	0.83	9.09	2,648	0.29
Mean	1.09	9.58	2,509	0.40
SEM	0.21	0.97	307	0.04
Atorvastatin				
P1	0.00	3.82	644	0.49
P2	2.42	10.19	2,432	0.44
P3	0.34	5.24	1,062	0.52
P4	0.24	8.68	2,082	0.46
P5	1.40	6.67	1,001	0.58
P6	0.88	6.36	1,818	0.37
P7	0.00	6.88	2,001	0.25
P8	0.53	11.21	2,467	0.37
Mean	0.73	7.38	1,688 ^{a,b}	0.44 ^b
SEM	0.29	0.88	246	0.04
Fenofibrate				
P1	0.00	7.65	1,317	0.48
P2	0.00	12.37	3,147	0.33
P3	4.27	7.14	3,028	0.37
P5	2.03	7.55	1,707	0.40
P6	1.47	8.04	2,979	0.29
P7	1.92	7.84	3,461	0.21
P8	1.72	8.68	3,209	0.25
Mean	1.63	8.47	2,693	0.33 ^c
SEM	0.55	0.67	313	0.03

^a $P < 0.01$ versus baseline.^b $P < 0.05$ versus fenofibrate (Wilcoxon test).^c $P < 0.05$ versus baseline.

TABLE 6. HDL apoA-I kinetics in controls and in patients at baseline and during atorvastatin and fenofibrate therapy

Subjects	Production	Pool	FCR
	mg/kg/day	mg	pools/day
Controls			
C1	16.2	3,763	0.30
C2	8.3	3,248	0.18
C3	7.1	3,093	0.17
C4	6.8	4,146	0.14
C5	6.3	2,602	0.15
C6	19.1	5,266	0.28
C7	14.0	4,016	0.26
C8	14.1	4,768	0.21
Mean	11.49	3,863	0.21
SEM	1.75	312	0.02
Patients			
Baseline			
P1	9.7	2,810	0.28
P2	11.6	4,009	0.24
P3	10.4	3,982	0.25
P4	15.0	4,181	0.38
P5	13.3	3,971	0.24
P6	10.4	4,176	0.22
P7	9.5	3,090	0.23
P8	14.3	3,074	0.37
Mean	11.78	3,662	0.28
SEM	0.76	200	0.02
Atorvastatin			
P1	9.6	2,849	0.27
P2	19.3	4,233	0.38
P3	14.8	4,744	0.30
P4	10.3	4,026	0.27
P5	11.4	4,076	0.20
P6	11.6	4,552	0.23
P7	9.1	3,056	0.23
P8	8.4	3,173	0.20
Mean	11.81 ^a	3,839	0.26
SEM	1.28	254	0.02
Fenofibrate			
P1	16.0	3,384	0.38
P2	16.4	4,380	0.31
P3	20.7	4,256	0.47
P5	20.1	4,484	0.32
P6	14.5	4,956	0.26
P7	13.2	2,990	0.32
P8	12.3	3,018	0.31
Mean	16.17 ^b	3,924	0.34 ^b
SEM	1.22	296	0.03

^a $P < 0.05$ versus fenofibrate (Wilcoxon test).^b $P < 0.05$ versus controls (Kruskal-Wallis test).

37). In accordance, the decrease in LDL apoB synthesis found by us and other investigators is likely to result from the enhanced removal of LDL precursors via the LDL receptor pathway (38–40). Interestingly, the extent of the atorvastatin-associated increase in LDL apoB catabolism in our study was clearly correlated with the LDL apoB concentration and the LDL apoB turnover rate at baseline (Fig. 4). Thus, subjects with the highest LDL apoB concentrations and the lowest catabolic rates experienced the most pronounced increase in LDL apoB catabolism during atorvastatin treatment. This suggests that the extent of increase in LDL catabolism secondary to statins is dependent on the baseline metabolic state and offers an explanation for the previous observation of a correlation between baseline LDL cholesterol concentrations and the

extent of their decrease during simvastatin therapy (41). However, two additional mechanisms contributed to the lipid-lowering mechanism of atorvastatin in our study. First, atorvastatin-treated subjects demonstrated a 2.4-fold increase in the delipidation of VLDL₁ to VLDL₂. This finding is in agreement with recently reported results from other apoB kinetic studies (36, 37). Removal of triglycerides from circulating triglyceride-rich lipoproteins is mediated by lipoprotein lipase (LPL). The activity of this enzyme is critically dependent on its cofactor apoC-II, whereas apoC-III has been found to be inhibitory (42). Simvastatin has recently been described to increase LPL activity and mRNA in rat heart and adipose tissue, and this was ascribed to a decrease of liver apolipoprotein C-III (apoC-III) mRNA expression (43). In analogy, it can be speculated that the triglyceride-lowering effect of atorva-

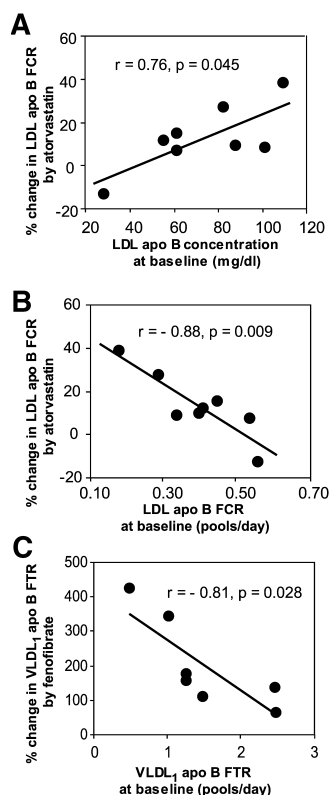


Fig. 4. The response of LDL apoB catabolism to atorvastatin was most pronounced in patients with high LDL apoB concentrations (A) and low LDL catabolic rates (B) at baseline. Patients with low VLDL₁ fractional transfer rates (FTR) at baseline showed the greatest response to fenofibrate therapy (C). FCR, fractional catabolic rate.

statin in our patients was at least in part secondary to an increase in LPL activity resulting from decreased apoC-III levels. This may result from the activation of the nuclear receptor PPAR α , which has recently been implicated as a mediator of statin effects on lipid metabolism (44). Second, hepatic secretion of VLDL₁ apoB, which accounted for 78% of total apoB secretion in the basal state, decreased in six patients, a finding in agreement with some, but not all, previous apoB kinetic studies of patients with mixed hyperlipidemia (34, 36, 39, 40). As recently reviewed in detail, these discrepancies may be explained by different underlying metabolic conditions in the subjects enrolled in these studies (40). Hepatic apoB secretion has been shown to be largely substrate-driven (45). The finding of a decreased VLDL₁ cholesterol ester content (3.9 vs. 6.6%; $P < 0.001$; data not shown) supports the view that a decrease of hepatocellular cholesterol levels secondary to statin treatment may limit the intracellular availability of cholesterol esters to form mature lipoproteins and hence impair hepatic lipoprotein secretion (46). Therefore, the profound triglyceride- and cholesterol-lowering effects of atorvastatin in mixed hyperlipidemia result from both the decreased production and accelerated breakdown and removal of triglyceride-rich lipoproteins, whereas maintaining a high rate of LDL catabolism prevents an increase in LDL after enhanced delipidation of

its precursors. The absence of an effect of atorvastatin on apoA-I kinetics agrees with findings from previous kinetic studies of normolipidemic subjects and patients with the metabolic syndrome (37, 47). Therefore, the beneficial effects of atorvastatin on HDL metabolism seem to be secondary to its effect on the metabolism of triglyceride-rich lipoproteins and the resulting decrease of core lipid exchange between these lipoprotein fractions.


Therapy with fibrates such as fenofibrate has consistently been shown to decrease plasma VLDL cholesterol and triglyceride levels and to increase HDL cholesterol, thereby favorably influencing the cardiovascular risk profile. With regard to LDL cholesterol, both increased and decreased plasma concentrations have been observed, and the fibrate effect seemed to be largely dependent on the pretreatment lipid phenotype (48). Fibrate effects on lipid metabolism result from the activation of the nuclear receptor PPAR α , an important regulator of genes involved in the oxidation of fatty acids, lipogenesis, intravascular catabolism of triglyceride-rich lipoproteins, and reverse cholesterol transport (16). Decreased hepatic triglyceride production secondary to the decreased availability of fatty acids and accelerated delipidation and removal of VLDL attributable to enhanced LPL activity have been found to be consistent effects of fibrates in vivo (49–51). Previous kinetic studies in patients with endogenous hypertriglyceridemia or the metabolic syndrome using either bezafibrate or fenofibrate reported an increase of VLDL apolipoprotein catabolism but no change in VLDL apoB production (37, 52).

The kinetic data obtained in this study demonstrate that the triglyceride-lowering effect of fenofibrate results from both enhanced direct removal and delipidation of VLDL₁. The VLDL₁ transfer rate during fenofibrate therapy was inversely correlated with the baseline status, indicating that fenofibrate was most effective in patients with a low VLDL₁ turnover at baseline (Fig. 4). Furthermore, VLDL₂ particles were more rapidly converted to IDL and then removed from the circulation. ApoB production in the VLDL₁ or VLDL₂ density range was unaffected. Because the lipid composition of VLDL remained unaltered, this suggests that fenofibrate did not interfere with the hepatic secretion of triglyceride-rich lipoproteins in our patients. Whether the lack of a fenofibrate effect on hepatic lipoprotein production is substance specific or a consequence of the underlying metabolic defect in our patients is unclear.

Regarding LDL metabolism, previous kinetic studies using radioisotopes clearly suggested that the effects of fibrates depend on the underlying lipid disorder. In subjects with hypercholesterolemia, fenofibrate, bezafibrate, and ciprofibrate decreased LDL cholesterol by enhancing receptor-mediated clearance (53–55). In moderate to severe hypertriglyceridemic subjects, however, LDL is relatively triglyceride rich and its catabolic rate is often markedly increased. Treatment with fenofibrate results in a normalization of LDL composition, a decrease in the LDL FCR, and thereby an increase of the LDL cholesterol plasma level. Fenofibrate also induces a shift from small,

dense to larger, buoyant LDLs (56). These observations were all confirmed in the present study.

Fenofibrate resulted in an increase of apoA-I catabolism that was more than compensated for by a concomitant increase in the rate of apoA-I production. As a net result, apoA-I and thereby HDL turnover in plasma was increased, which conceivably is beneficial with regard to the role of HDL in reverse cholesterol transport. These findings are in agreement with the results of some, but not all, studies reporting apoA-I kinetic data in patients treated with various fibrates (37, 52, 53, 57). Differences between the underlying lipid disorders, the fibrate derivatives used, and the study designs may explain these discrepancies. Direct transcriptional effects of PPAR α activation on the hepatic apoA-I gene seem to account for the increased hepatic apoA-I production observed (16). The increase in apoA-I catabolism found in this and other studies may be explained by the enhanced intravascular remodeling of HDL₂ to small, dense, more easily catabolized HDL₃ secondary to the activation of hepatic lipase by fenofibrate (58). In analogy to the findings obtained during atorvastatin treatment, the marked reduction in the concentrations of triglyceride-rich lipoproteins were associated with a decrease in HDL triglyceride content and reciprocal changes in HDL cholesterol levels.

In summary, the results of this study demonstrate that in patients with moderate to severe mixed hyperlipidemia attributable to both the overproduction and delayed removal of VLDL, atorvastatin and fenofibrate are equally effective at decreasing plasma triglycerides. Both drugs accelerate the delipidation of VLDL₁, presumably by increasing LPL activity. Additionally, atorvastatin enhances the removal of VLDL₂ from plasma, and fenofibrate increases the direct catabolism of VLDL₁. Decreased apoB and LDL cholesterol plasma concentrations with atorvastatin therapy can be ascribed to increased rates of LDL catabolism and the removal of LDL precursors from the circulation, suggesting that statins may be particularly efficacious in subjects with increased triglycerides and LDL cholesterol levels. In contrast, fenofibrate did not decrease LDL cholesterol effectively, although it induced a normalization of the LDL subfraction distribution. Whereas atorvastatin only normalized HDL composition but left apoA-I metabolism largely unaffected, fenofibrate increased HDL cholesterol concentrations and plasma turnover by increasing the rates of apoA-I production and catabolism. 

This study was supported by a grant from Warner Lambert Parke Davis (Baar, Switzerland).

REFERENCES

1. Grundy, S. M. 1995. Atherogenic dyslipidemia: lipoprotein abnormalities and implications for therapy. *Am. J. Cardiol.* **75**: 45B–52B.
2. Frick, M. H., O. Elo, K. Haapa, O. P. Heinonen, P. Heinsalmi, P. Helo, J. K. Huttunen, P. Kaitaniemi, P. Koskinen, and V. Manninen. 1987. Helsinki Heart Study: primary-prevention trial with

- gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N. Engl. J. Med.* **317**: 1237–1245.
3. The Scandinavian Simvastatin Survival Study Group. 1994. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet.* **344**: 1383–1389.
4. Shepherd, J., S. M. Cobbe, I. Ford, C. G. Isles, A. R. Lorimer, P. W. MacFarlane, J. H. McKillop, and C. J. Packard. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N. Engl. J. Med.* **333**: 1301–1307.
5. Sacks, F. M., M. A. Pfeffer, L. A. Moye, J. L. Rouleau, J. D. Rutherford, T. G. Cole, L. Brown, J. W. Warnica, J. M. Arnold, C. C. Wun, B. R. Davis, and E. Braunwald. 1996. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N. Engl. J. Med.* **335**: 1001–1009.
6. The Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) Study Group. 1998. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N. Engl. J. Med.* **339**: 1349–1357.
7. Rubins, H. B., S. J. Robins, D. Collins, C. L. Fye, J. W. Anderson, M. B. Elam, F. H. Faas, E. Linares, E. J. Schaefer, G. Schectman, T. J. Wilt, and J. Wittes. 1999. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N. Engl. J. Med.* **341**: 410–418.
8. The Heart Protection Study Collaborative Group. 2002. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet.* **360**: 7–22.
9. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2001. *J. Am. Med. Assoc.* **285**: 2486–2497.
10. Ma, P. T., G. Gil, T. C. Sudhof, D. W. Bilheimer, J. L. Goldstein, and M. S. Brown. 1986. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. *Proc. Natl. Acad. Sci. USA.* **83**: 8370–8374.
11. Bakker-Arkema, R. G., M. H. Davidson, R. J. Goldstein, J. Davignon, J. L. Isaacs, S. R. Weiss, L. M. Keilson, W. V. Brown, V. T. Miller, L. J. Shurzinske, and D. M. Black. 1996. Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. *J. Am. Med. Assoc.* **275**: 128–133.
12. Naoumova, R. P., S. Dunn, L. Rallidis, O. Abu-Muhana, C. Neuwirth, N. B. Rendell, G. W. Taylor, and G. R. Thompson. 1997. Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J. Lipid Res.* **38**: 1496–1500.
13. Burnett, J. R., L. J. Wilcox, D. E. Telford, S. J. Kleinstiver, P. H. Barrett, R. S. Newton, M. W. Huff, and P. Barrett. 1997. Inhibition of HMG-CoA reductase by atorvastatin decreases both VLDL and LDL apolipoprotein B production in miniature pigs. *Arterioscler. Thromb. Vasc. Biol.* **17**: 2589–2600.
14. Marais, A. D., R. P. Naoumova, J. C. Firth, C. Penny, C. K. Neuwirth, and G. R. Thompson. 1997. Decreased production of low density lipoprotein by atorvastatin after apheresis in homozygous familial hypercholesterolemia. *J. Lipid Res.* **38**: 2071–2080.
15. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37**: 907–925.
16. Staels, B., J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, and J. C. Fruchart. 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation.* **98**: 2088–2093.
17. Packard, C. J., T. Demant, J. P. Stewart, D. Bedford, M. J. Caslake, G. Schwertfeger, A. Bedynek, J. Shepherd, and D. Seidel. 2000. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J. Lipid Res.* **41**: 305–318.
18. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations, Volume 1: Lipid and Lipoprotein Analysis. DHES Publications No. (NIH), Bethesda, MD. 75–268.
19. Demant, T., C. J. Packard, H. Demmelmair, P. Stewart, A. Bedynek,

- D. Bedford, D. Seidel, and J. Shepherd. 1996. Sensitive methods to study human apolipoprotein B metabolism using stable isotope-labeled amino acids. *Am. J. Physiol.* **270**: E1022-E1036.
20. Schmitz, M., G. M. Michl, R. Walli, J. Bogner, A. Bedynek, D. Seidel, F. D. Goebel, and T. Demant. 2001. Alterations of apolipoprotein B metabolism in HIV-infected patients with antiretroviral combination therapy. *J. Acquired Immune Defic. Syndr.* **26**: 225-235.
 21. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The Isolation and Quantitation Analysis of Serum Lipoproteins. Wiley-Interscience, New York. 181-274.
 22. Egusa, G., D. W. Brady, S. M. Grundy, and B. V. Howard. 1983. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24**: 1261-1267.
 23. Lowry, O., N. Rosebrough, A. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
 24. Gylling, H., G. L. Vega, and S. M. Grundy. 1992. Physiologic mechanisms for reduced apolipoprotein A-I concentrations associated with low levels of high density lipoprotein cholesterol in patients with normal plasma lipids. *J. Lipid Res.* **33**: 1527-1539.
 25. Janus, E. D., A. M. Nicoll, P. R. Turner, P. Magill, and B. Lewis. 1980. Kinetic bases of the primary hyperlipidaemias: studies of apolipoprotein B turnover in genetically defined subjects. *Eur. J. Clin. Invest.* **10**: 161-172.
 26. Kissebah, A. H., S. Alfarsi, and P. W. Adams. 1981. Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in man: normolipemic subjects, familial hypertriglyceridemia and familial combined hyperlipidemia. *Metabolism.* **30**: 856-868.
 27. Teng, B., A. D. Sniderman, A. K. Soutar, and G. R. Thompson. 1986. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J. Clin. Invest.* **77**: 663-672.
 28. Venkatesan, S., P. Cullen, P. Pacy, D. Halliday, and J. Scott. 1993. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler. Thromb.* **13**: 1110-1118.
 29. Lamarche, B., S. Rashid, and G. F. Lewis. 1999. HDL metabolism in hypertriglyceridemic states: an overview. *Clin. Chim. Acta.* **286**: 145-161.
 30. Murakami, T., S. Michelagnoli, R. Longhi, G. Gianfranceschi, F. Pazzucconi, L. Calabresi, C. R. Sirtori, and G. Franceschini. 1995. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. *Arterioscler. Thromb. Vasc. Biol.* **15**: 1819-1828.
 31. Lamarche, B., K. D. Uffelman, A. Carpentier, J. S. Cohn, G. Steiner, P. H. Barrett, and G. F. Lewis. 1999. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J. Clin. Invest.* **103**: 1191-1199.
 32. Vega, G. L., and S. M. Grundy. 1991. Influence of lovastatin therapy on metabolism of low density lipoproteins in mixed hyperlipidaemia. *J. Intern. Med.* **230**: 341-350.
 33. Parhofer, K. G., P. H. Barrett, J. Dunn, and G. Schonfeld. 1993. Effect of pravastatin on metabolic parameters of apolipoprotein B in patients with mixed hyperlipoproteinemia. *Clin. Invest.* **71**: 939-946.
 34. Aguilar-Salinas, C. A., P. Hugh, R. Barrett, J. Pulai, X. L. Zhu, and G. Schonfeld. 1997. A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism. Kinetics of apolipoprotein B during placebo and pravastatin therapy. *Arterioscler. Thromb. Vasc. Biol.* **17**: 72-82.
 35. Chan, D. C., G. F. Watts, P. H. Barrett, L. J. Beilin, T. G. Redgrave, and T. A. Mori. 2002. Regulatory effects of HMG CoA reductase inhibitor and fish oils on apolipoprotein B-100 kinetics in insulin-resistant obese male subjects with dyslipidemia. *Diabetes.* **51**: 2377-2386.
 36. Forster, L. F., G. Stewart, D. Bedford, J. P. Stewart, E. Rogers, J. Shepherd, C. J. Packard, and M. J. Caslake. 2002. Influence of atorvastatin and simvastatin on apolipoprotein B metabolism in moderate combined hyperlipidemic subjects with low VLDL and LDL fractional clearance rates. *Atherosclerosis.* **164**: 129-145.
 37. Watts, G. F., P. H. Barrett, J. Ji, A. P. Serone, D. C. Chan, K. D. Croft, F. Loehrer, and A. G. Johnson. 2003. Differential regulation of lipoprotein kinetics by atorvastatin and fenofibrate in subjects with the metabolic syndrome. *Diabetes.* **52**: 803-811.
 38. Vega, G. L., R. M. Krauss, and S. M. Grundy. 1990. Pravastatin therapy in primary moderate hypercholesterolaemia: changes in metabolism of apolipoprotein B-containing lipoproteins. *J. Intern. Med.* **227**: 81-94.
 39. Arad, Y., R. Ramakrishnan, and H. N. Ginsberg. 1992. Effects of lovastatin therapy on very-low-density lipoprotein triglyceride metabolism in subjects with combined hyperlipidemia: evidence for reduced assembly and secretion of triglyceride-rich lipoproteins. *Metabolism.* **41**: 487-493.
 40. Cuchel, M., E. J. Schaefer, J. S. Millar, P. J. Jones, G. G. Dolnikowski, C. Vergani, and A. H. Lichtenstein. 1997. Lovastatin decreases de novo cholesterol synthesis and LDL apo B-100 production rates in combined-hyperlipidemic males. *Arterioscler. Thromb. Vasc. Biol.* **17**: 1910-1917.
 41. Miserez, A. R., F. A. Rossi, and U. Keller. 1994. Prediction of the therapeutic response to simvastatin by pretreatment lipid concentrations in 2082 subjects. *Eur. J. Clin. Pharmacol.* **46**: 107-114.
 42. Wang, C. S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75**: 384-390.
 43. Schoonjans, K., J. Peinado-Onsurbe, J. C. Fruchart, A. Tailleux, C. Fievet, and J. Auwerx. 1999. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors reduce serum triglyceride levels through modulation of apolipoprotein C-III and lipoprotein lipase. *FEBS Lett.* **452**: 160-164.
 44. Martin, G., H. Duez, C. Blanquart, V. Berezowski, P. Poulain, J. C. Fruchart, J. Najib-Fruchart, C. Glineur, and B. Staels. 2001. Statin-induced inhibition of the Rho-signaling pathway activates PPAR α and induces HDL apoA-I. *J. Clin. Invest.* **107**: 1423-1432.
 45. Ginsberg, H. N. 1995. Synthesis and secretion of apolipoprotein B from cultured liver cells. *Curr. Opin. Lipidol.* **6**: 275-280.
 46. Thompson, G. R., R. P. Naumova, and G. F. Watts. 1996. Role of cholesterol in regulating apolipoprotein B secretion by the liver. *J. Lipid Res.* **37**: 439-447.
 47. Schaefer, J. R., H. Schweer, K. Ikewaki, H. Stracke, H. J. Seyberth, H. Kaffarnik, B. Maisch, and A. Steinmetz. 1999. Metabolic basis of high density lipoproteins and apolipoprotein A-I increase by HMG-CoA reductase inhibition in healthy subjects and a patient with coronary artery disease. *Atherosclerosis.* **144**: 177-184.
 48. Grundy, S. M., and G. L. Vega. 1987. Fibric acids: effects on lipids and lipoprotein metabolism. *Am. J. Med.* **83**: 9-20.
 49. Kissebah, A. H., P. W. Adams, P. Harrigan, and V. Wynn. 1974. The mechanism of action of clofibrate and tetranicotinoylfructose (Bradilan) on the kinetics of plasma free fatty acid and triglyceride transport in type IV and type V hypertriglyceridaemia. *Eur. J. Clin. Invest.* **4**: 163-174.
 50. Kesaniemi, Y. A., and S. M. Grundy. 1984. Influence of gemfibrozil and clofibrate on metabolism of cholesterol and plasma triglycerides in man. *J. Am. Med. Assoc.* **251**: 2241-2246.
 51. Ginsberg, H. N. 1987. Changes in lipoprotein kinetics during therapy with fenofibrate and other fibric acid derivatives. *Am. J. Med.* **83**: 66-70.
 52. Shepherd, J., C. J. Packard, J. M. Stewart, R. F. Atmeh, R. S. Clark, D. E. Boag, K. Carr, A. R. Lorimer, D. Ballantyne, H. G. Morgan, T. D. V. Lawrie. 1984. Apolipoprotein A and B (Sf 100-400) metabolism during bezafibrate therapy in hypertriglyceridemic subjects. *J. Clin. Invest.* **74**: 2164-2177.
 53. Malmendier, C. L., and C. Delcroix. 1985. Effects of fenofibrate on high and low density lipoprotein metabolism in heterozygous familial hypercholesterolemia. *Atherosclerosis.* **55**: 161-169.
 54. Stewart, J. M., C. J. Packard, A. R. Lorimer, D. E. Boag, and J. Shepherd. 1982. Effects of bezafibrate on receptor-mediated and receptor-independent low density lipoprotein catabolism in type II hyperlipoproteinaemic subjects. *Atherosclerosis.* **44**: 355-365.
 55. Gaw, A., C. J. Packard, M. J. Caslake, B. A. Griffin, G. M. Lindsay, J. Thomson, B. D. Vallance, D. Wosornu, and J. Shepherd. 1994. Effects of ciprofibrate on LDL metabolism in man. *Atherosclerosis.* **108**: 137-148.
 56. Shepherd, J., M. J. Caslake, A. R. Lorimer, B. D. Vallance, and C. J. Packard. 1985. Fenofibrate reduces low density lipoprotein catabolism in hypertriglyceridemic subjects. *Arteriosclerosis.* **5**: 162-168.
 57. Saku, K., P. S. Gartside, B. A. Hynd, and M. L. Kashyap. 1985. Mechanism of action of gemfibrozil on lipoprotein metabolism. *J. Clin. Invest.* **75**: 1702-1712.
 58. Simpson, H. S., C. M. Williamson, T. Olivecrona, S. Pringle, J. Maclean, A. R. Lorimer, F. Bonnefous, Y. Bogaevsky, C. J. Packard, and J. Shepherd. 1990. Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis.* **85**: 193-202.