

Effects of different doses of atorvastatin on human apolipoprotein B-100, B-48, and A-I metabolism

Stefania Lamon-Fava,^{1,*} Margaret R. Diffenderfer,^{*} P. Hugh R. Barrett,[†] Aaron Buchsbaum,^{*} Nirupa R. Matthan,[§] Alice H. Lichtenstein,[§] Gregory G. Dolnikowski,^{**} Katalin Horvath,^{*} Bela F. Asztalos,^{*} Valeria Zago,^{*} and Ernst J. Schaefer^{*,††}

Lipid Metabolism Laboratory,^{*} Cardiovascular Nutrition Laboratory,[§] and Mass Spectrometry Laboratory,^{**} Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; School of Medicine and Pharmacology,[†] University of Western Australia, Perth, Australia; and Cardiovascular Research Laboratory,^{††} Friedman School of Nutrition Science and Policy at Tufts University and Tufts University School of Medicine, Boston, MA

Abstract Nine hypercholesterolemic and hypertriglyceridemic subjects were enrolled in a randomized, placebo-controlled, double-blind, crossover study to test the effect of atorvastatin 20 mg/day and 80 mg/day on the kinetics of apolipoprotein B-100 (apoB-100) in triglyceride-rich lipoprotein (TRL), intermediate density lipoprotein (IDL), and LDL, of apoB-48 in TRL, and of apoA-I in HDL. Compared with placebo, atorvastatin 20 mg/day was associated with significant reductions in TRL, IDL, and LDL apoB-100 pool size as a result of significant increases in fractional catabolic rate (FCR) without changes in production rate (PR). Compared with the 20 mg/day dose, atorvastatin 80 mg/day caused a further significant reduction in the LDL apoB-100 pool size as a result of a further increase in FCR. ApoB-48 pool size was reduced significantly by both atorvastatin doses, and this reduction was associated with nonsignificant increases in FCR. The lathosterol-campesterol ratio was decreased by atorvastatin treatment, and changes in this ratio were inversely correlated with changes in TRL apoB-100 and apoB-48 PR. No significant effect on apoA-I kinetics was observed at either dose of atorvastatin. **Our data indicate that atorvastatin reduces apoB-100- and apoB-48-containing lipoproteins by increasing their catabolism and has a dose-dependent effect on LDL apoB-100 kinetics. Atorvastatin-mediated changes in cholesterol homeostasis may contribute to apoB PR regulation.**—Lamon-Fava, S., M. R. Diffenderfer, P. H. R. Barrett, A. Buchsbaum, N. R. Matthan, A. H. Lichtenstein, G. G. Dolnikowski, K. Horvath, B. F. Asztalos, V. Zago, and E. J. Schaefer. **Effects of different doses of atorvastatin on human apolipoprotein B-100, B-48, and A-I metabolism.** *J. Lipid Res.* 2007. 48: 1746–1753.

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Atorvastatin is a lipid-lowering medication that belongs to the family of HMG-CoA reductase inhibitors.

The primary effect of this family of medications is to decrease apolipoprotein B (apoB)-containing lipoproteins, especially LDLs, by inhibiting the rate-limiting step in the cholesterol biosynthesis pathway. The decrease of plasma LDL-cholesterol (-C) levels by statins has been attributed to a reduction in hepatic apoB secretion in some studies (1, 2) and to an enhanced LDL receptor-mediated clearance in other studies (3–6). Atorvastatin, one of the most potent statins, has a dose-dependent effect on LDL-C, with the 80 mg/day dose decreasing plasma LDL-C by ~10–14% more than the 20 mg/day dose (7, 8). It is not known whether the further reduction in LDL-C levels achieved at higher doses of atorvastatin is mediated by the same mechanism that causes the reduction at lower doses.

Atorvastatin is also known to modestly increase (5%) plasma levels of HDL-C at lower doses, but the administration of higher doses (80 mg/day) is associated with a blunting in the HDL-C increase (8), accompanied in some cases by a reduction in apoA-I levels, the main protein in HDL (9).

The objective of our study was to assess the effect of two different doses of atorvastatin on the kinetic parameters of apoB-100 in triglyceride-rich lipoprotein (TRL), intermediate density lipoprotein (IDL), and LDL, of apoB-48 in chylomicrons, and of apoA-I in HDL in the nonfasting state in healthy subjects with mixed hyperlipidemia. Our purpose was to determine the contribution of changes in apolipoprotein kinetic parameters to changes in LDL-C and HDL-C levels. In addition, we wanted to assess the contribution of cholesterol synthesis inhibition and intestinal cholesterol absorption modification by atorvastatin on apolipoprotein kinetic parameters.

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¹To whom correspondence should be addressed.
e-mail: stefania.lamon-fava@tufts.edu

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Subjects

Nine subjects with combined hyperlipidemia were enrolled in this study (mean age \pm SD, 55 ± 8 years; body mass index, 28.07 ± 3.15 kg/m²). Five were men and four were postmenopausal women not on hormone replacement therapy. Plasma lipid criteria for enrollment in the study were as follows: plasma triglyceride (TG) levels ≥ 150 mg/dl, LDL-C levels ≥ 160 mg/dl, and low HDL-C levels (≤ 40 mg/dl in men and ≤ 50 mg/dl in women). Exclusion criteria were as follows: age < 40 years, smoking, thyroid dysfunction, liver or kidney disease, diabetes mellitus, stroke, myocardial infarction in the past 6 months, and current use of medications known to affect lipid metabolism. The study protocol was approved by the Institutional Review Board of Tufts University-New England Medical Center. Study candidates provided written informed consent.

Study design

Subjects were instructed to follow the therapeutic lifestyle changes diet, as recommended by the National Cholesterol Education Program Adult Treatment Panel III ($<30\%$ of calories as total fat, $<7\%$ saturated fat, <200 mg/day cholesterol) (10) throughout the study. After 3 weeks of a lead-in therapeutic lifestyle changes diet phase, subjects were randomized to treatment. The study had a randomized, double-blind, crossover design and consisted of three treatment phases: placebo, atorvastatin 20 mg/day, and atorvastatin 80 mg/day. Each phase lasted 8 weeks. Phases were separated by a 4 week washout period. During weeks 7 and 8 of each phase, a blood sample was obtained after a 12 h fast for the determination of plasma lipid levels. Plasma was separated at 1,000 g for 30 min at $+4^\circ\text{C}$ and stored at -70°C until analyzed.

During week 8 of each phase, subjects underwent a primed-constant infusion with deuterated leucine [$5,5,5\text{-}^2\text{H}_3$]L-leucine, as described previously (2, 11, 12). Briefly, subjects were admitted to the General Clinical Research Center of Tufts University-New England Medical Center to undergo a primed-constant infusion of 10 $\mu\text{mol/kg}$ body weight/h [$5,5,5\text{-}^2\text{H}_3$]L-leucine (C/D/N Isotopes, Inc., Pointe-Claire, Quebec, Canada) for 15 h. Subjects were fed hourly for 20 h with small identical meals starting 5 h before and throughout the infusion. Blood samples were collected into tubes containing EDTA (0.15%) just before the infusion (time 0) and at the following times during the infusion: 30, 35, 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 14, and 15 h.

Plasma lipid determinations and lipoprotein isolation

Lipids were measured both in fasting (weeks 7 and 8) and fed (hours 0, 3, and 6 of infusion) plasma samples. Plasma total cholesterol (TC) and TG levels were measured by automated enzymatic assays (13). Plasma LDL-C and HDL-C concentrations were measured directly with kits from Equal Diagnostics (Exton, PA) and Roche Diagnostics (Indianapolis, IN), respectively.

Five milliliters of plasma from each infusion time point were subjected to sequential ultracentrifugation in a Beckman ultracentrifuge (Beckman, Palo Alto, CA) for the isolation of TRL ($d < 1.006$ g/ml), IDL ($d = 1.006\text{--}1.019$ g/ml), LDL ($d = 1.019\text{--}1.063$ g/ml), and HDL ($d = 1.063\text{--}1.21$ g/ml) fractions, as described previously (14). The concentration of apoB in plasma and in the TRL and IDL fractions was measured with an ELISA using immunoaffinity-purified polyclonal antibodies (BioDesign, Saco, ME). The coefficient of variation for the apoB assay was $<5\%$. The concentration of LDL apoB was calculated as follows: plasma apoB $-$ (TRL apoB + IDL apoB). To assess the apoB-48 concentration, the TRL fraction was subjected to SDS-PAGE and stained with 0.1% Coomassie Blue R-250. The relative proportion

of apoB-100 and apoB-48 was assessed by densitometric scanning (15). Plasma apoA-I concentrations were measured using an immunoturbidimetric assay, reagents, and calibrators from Wako Diagnostics (Richmond, VA).

Cholesteryl ester transfer protein activity was measured as the rate of decrease in HDL cholesteryl esters (nmol/ml/h) at 37°C , and LCAT activity was measured as the rate of free cholesterol disappearance from HDL (nmol/ml/h) at 37°C , according to the method described by Ogawa and Fielding (16).

Apolipoprotein isotopic enrichment and kinetic analysis

TRL, IDL, LDL, and HDL fractions were subjected to gradient SDS-PAGE, and the proteins were transferred to a Westran S polyvinylidene difluoride membrane (Whatman Schleicher and Schuell BioScience, Sanford, ME) using a Tris-glycine-methanol buffer system (17). The apoB-48 band in TRL, the apoB-100 band in TRL, IDL, and LDL, and the apoA-I band in HDL were visualized with Coomassie Blue R-250, excised from the membrane, hydrolyzed in 12 N HCl at 110°C for 24 h, and evaporated to dryness. Amino acids were converted to heptafluorobutyramide derivatives and analyzed on an Agilent Technologies 6890/5973N gas chromatograph/mass spectrometer. Selected ion monitoring at m/z 349 (derivatized leucine-HF) and m/z 352 (derivatized leucine- d_3 -HF) was used to determine the areas under the curve of each ion. Mole percentage enrichment (leucine- d_3 /leucine) for each sample was calculated from the areas under the curve and converted to a tracer-tracee ratio (percentage) as described previously (18). The Simulation Analysis and Modeling II (SAAM II) program (Seattle, WA) was used to calculate the apoB-100 and apoB-48 fractional catabolic rates (FCRs) using multicompartmental models, as described previously (15, 19). SAAM II was also used to calculate apoA-I FCR using a multicompartmental model described previously (12). ApoB-100, apoB-48, and apoA-I production rates (PRs) were determined by the following formula:

$$\text{PR (mg/kg/day)} = [\text{FCR (pools/day)} \times \text{apolipoprotein concentration (mg/l)} \times \text{plasma volume (liters)}] / \text{body weight (kg)}$$

Plasma volume was estimated as 4.5% of body weight.

Plasma sterol determinations

Plasma concentrations of lathosterol, a precursor in the biosynthesis of cholesterol, and of the plant sterols campesterol and β -sitosterol were assessed in eight of the nine subjects using a gas chromatography method as described previously (20). Concentrations of the plant sterols campesterol and β -sitosterol are used as markers of intestinal cholesterol absorption (21). Because these noncholesterol sterols are transported in plasma by lipoproteins, their concentrations are expressed relative to the concentration of plasma TC to correct for the different number of lipoprotein acceptor particles during the placebo and treatment phases.

Statistical analyses

The SPSS statistical package (version 14; SPSS, Chicago, IL) was used for statistical analyses. Significant differences in means between phases were assessed by paired Student's *t*-tests. Spearman correlations were used in correlation analyses. $P < 0.05$ was considered significant.

RESULTS

Plasma TC, LDL-C, and TG concentrations were decreased significantly by treatment with atorvastatin 20 mg/

day, compared with placebo, in both the fasted and non-fasted states ($P < 0.01$) (Table 1). Increasing the daily dose of atorvastatin to 80 mg was associated with further significant reductions only in plasma TC and LDL-C concentrations, compared with the 20 mg dose ($P < 0.05$) (Table 1). Plasma HDL-C levels were unaffected by treatment in the fasted state but were increased significantly by 20 mg of atorvastatin, compared with placebo, and decreased significantly by 80 mg of atorvastatin, compared with 20 mg of atorvastatin ($P < 0.05$), in the fed state (Table 1).

The effect of atorvastatin on plasma apolipoprotein concentrations in the fed state is shown in Table 2. Relative to placebo, total apoB concentrations were decreased significantly during treatment with both doses of atorvastatin, mostly accounted for by reductions in LDL apoB-100 ($P < 0.0001$). Significant reductions were also observed for IDL apoB-100 ($P < 0.01$). TRL apoB-100, however, was reduced significantly only by the 20 mg daily regimen ($P < 0.05$). The concentration of apoB-48 in TRL was decreased significantly by both doses of atorvastatin, compared with placebo ($P < 0.05$) (Table 2). Atorvastatin had no significant effect on plasma apoA-I concentrations.

Figure 1 shows the leucine tracer-tracee ratios and model-predicted values in TRL, IDL, and LDL apoB-100 and in TRL apoB-48 during the placebo and atorvastatin phases. During atorvastatin 20 mg/day, the pool sizes of TRL, IDL, and LDL apoB-100 were reduced significantly, mostly attributable to significant increases in their respective FCRs (Table 3). Despite the lack of a significant effect of atorvastatin 80 mg/day on TRL apoB-100 pool size, the TRL apoB-100 FCR was increased significantly. Treatment with atorvastatin 80 mg/day was also associated with a significant reduction in IDL and LDL apoB-100 pool size, attributable to an increase in IDL and LDL apoB-100 FCR. In addition, atorvastatin 80 mg/day decreased LDL pool size and increased LDL apoB-100 FCR significantly more than atorvastatin 20 mg/day ($P < 0.05$) (Table 3). No significant effect of either dose of atorva-

statin was observed on TRL, IDL, and LDL apoB-100 PRs. The conversion rate of TRL apoB-100 to IDL apoB-100 did not differ during the placebo, atorvastatin 20 mg/day, and atorvastatin 80 mg/day phases ($45 \pm 20\%$, $40 \pm 19\%$, and $41 \pm 14\%$, respectively; $P = 0.5$). LDL apoB-100 was derived via two pathways, the rapid conversion of TRL to LDL and the conversion of IDL to LDL. The proportion of LDL apoB-100 derived from the conversion of IDL to LDL did not differ during the placebo, atorvastatin 20 mg/day, and atorvastatin 80 mg/day phases ($68 \pm 19\%$, $63 \pm 16\%$, and $63 \pm 19\%$, respectively; $P = 0.39$). Therefore, also the proportion of LDL apoB-100 derived directly from TRL apoB-100 did not change with treatment.

The significant reductions in TRL apoB-48 pool size observed during both doses of atorvastatin, compared with placebo, were associated with a nonsignificant trend toward higher TRL apoB-48 FCR (Table 3).

As predicted by the inhibitory effect of atorvastatin on cholesterol biosynthesis, plasma levels of lathosterol were reduced significantly by atorvastatin 20 mg/day and 80 mg/day, compared with placebo ($P < 0.005$) (Table 4). Plasma concentrations of the plant sterols campesterol and β -sitosterol, markers of intestinal cholesterol absorption, were increased significantly by treatment with both doses of atorvastatin ($P < 0.005$) (Table 4). The lathosterol-campesterol and lathosterol- β -sitosterol ratios, indicators of cholesterol homeostasis, were decreased significantly by both atorvastatin regimens. In addition, the atorvastatin 80 mg/day dose decreased these ratios significantly more than the 20 mg/day dose (Table 4).

During the atorvastatin 20 mg/day phase, relative to placebo, the changes in lathosterol-campesterol ratio were significantly associated with the changes in TRL apoB-100 PR and TRL apoB-48 PR ($r = -0.786$, $P < 0.02$ and $r = -0.762$, $P < 0.03$, respectively) (Fig. 2). During treatment with atorvastatin 80 mg/day, the change in lathosterol-campesterol ratio was significantly associated with the change in TRL apoB-48 PR ($r = -0.857$, $P < 0.02$), and a trend was observed for the association with

TABLE 1. Fasting and nonfasting plasma lipid and lipoprotein levels during the placebo and atorvastatin treatment phases in participating subjects (n = 9)

Parameter	Placebo	Atorvastatin, 20 mg/day	Atorvastatin, 80 mg/day	Percent Change ^a	Percent Change ^b	Percent Change ^c
Fasting						
TC	6.87 ± 0.62 (266 ± 24)	4.34 ± 0.59 (168 ± 23) ^d	3.80 ± 0.49 (147 ± 19) ^{d,e}	-36 ± 8	-44 ± 9	-12 ± 13
TG	2.89 ± 0.77 (255 ± 68)	1.80 ± 0.49 (159 ± 43) ^f	1.76 ± 0.49 (156 ± 43) ^d	-34 ± 24	-38 ± 12	5 ± 39
LDL-cholesterol	4.32 ± 0.83 (167 ± 32)	2.50 ± 0.54 (97 ± 21) ^d	1.91 ± 0.44 (74 ± 17) ^{d,e}	-41 ± 14	-55 ± 10	-23 ± 18
HDL-cholesterol	1.01 ± 0.18 (39 ± 7)	1.09 ± 0.18 (42 ± 7)	1.06 ± 0.18 (41 ± 7)	7 ± 10	4 ± 12	-3 ± 6
Nonfasting						
TC	6.54 ± 0.59 (253 ± 23)	3.98 ± 0.78 (154 ± 30) ^d	3.44 ± 0.54 (133 ± 21) ^{d,e}	-34 ± 12	-44 ± 9	-12 ± 15
TG	3.13 ± 0.73 (277 ± 65)	2.18 ± 0.59 (193 ± 52) ^f	2.15 ± 0.59 (190 ± 52) ^f	-28 ± 23	-30 ± 19	5 ± 32
LDL-cholesterol	3.88 ± 0.62 (150 ± 24)	2.15 ± 0.52 (83 ± 20) ^d	1.71 ± 0.39 (66 ± 15) ^{d,e}	-44 ± 14	-56 ± 8	-17 ± 22
HDL-cholesterol	0.91 ± 0.16 (35 ± 6)	1.01 ± 0.16 (39 ± 6) ^f	0.93 ± 0.13 (36 ± 5) ^e	14 ± 14	6 ± 11	-6 ± 6

TC, total cholesterol; TG, triglyceride. Values are expressed as means ± SD in mmol/l (mg/dl).

^a Percent change, atorvastatin 20 mg/day versus placebo.

^b Percent change, atorvastatin 80 mg/day versus placebo.

^c Percent change, atorvastatin 80 mg/day versus atorvastatin 20 mg/day.

^d Significantly different from placebo, $P < 0.0001$.

^e Significantly different from atorvastatin 20 mg/day, $P < 0.05$.

^f Significantly different from placebo, $P < 0.01$.

TABLE 2. Mean plasma apolipoprotein concentrations (nonfasting) during the leucine infusion studies

Parameter	Placebo	Atorvastatin, 20 mg/day	Atorvastatin, 80 mg/day	Percent Change ^a	Percent Change ^b	Percent Change ^c
	mg/dl	mg/dl	mg/dl			
Total apoB	111.4 ± 11.3	67.9 ± 10.9 ^d	60.5 ± 10.8 ^d	-39 ± 9	-45 ± 10	-10 ± 16
TRL apoB-100	9.9 ± 2.3	7.5 ± 1.6 ^e	8.1 ± 1.6	-22 ± 22	-14 ± 29	12 ± 29
IDL apoB-100	3.3 ± 0.9	2.1 ± 0.7 ^f	2.1 ± 0.4 ^f	-35 ± 18	-33 ± 22	8 ± 37
LDL apoB-100	97.6 ± 11.8	58.0 ± 10.2 ^d	49.9 ± 9.4 ^{d,g}	-40 ± 9	-49 ± 9	-13 ± 16
TRL apoB-48	0.65 ± 0.25	0.46 ± 0.15 ^e	0.42 ± 0.11 ^f	-23 ± 31	-31 ± 15	-2 ± 34
ApoA-I	103 ± 17	103 ± 16	97 ± 13	0 ± 11	-5 ± 12	-5 ± 9

ApoB, apolipoprotein B; IDL, intermediate density lipoprotein; TRL, triglyceride-rich lipoprotein.

^a Percent change, atorvastatin 20 mg/day versus placebo.

^b Percent change, atorvastatin 80 mg/day versus placebo.

^c Percent change, atorvastatin 80 mg/day versus atorvastatin 20 mg/day.

^d Significantly different from placebo, $P < 0.0001$.

^e Significantly different from placebo, $P < 0.05$.

^f Significantly different from placebo, $P < 0.01$.

^g Significantly different from atorvastatin 20 mg/day, $P < 0.05$.

TRL apoB-100 PR ($r = -0.657$, $P = 0.15$) (Fig. 2). Similar results were obtained when the lathosterol- β -sitosterol ratio was used (data not shown).

The changes in LDL-C levels during the 20 mg/day and 80 mg/day atorvastatin phases were inversely associated with the changes in LDL apoB-100 FCR ($r = -0.583$, $P = 0.09$ and $r = -0.667$, $P < 0.05$, respectively) (Fig. 3).

The rate of deuterated leucine incorporation into apoA-I was not affected by treatment (Fig. 4). The lower dose of atorvastatin did not change the metabolic parameters of HDL apoA-I. Treatment with 80 mg of atorvastatin was associated with a nonsignificant trend toward a reduction in apoA-I PR, compared with placebo (-5% ; $P = 0.186$) (Table 3).

LCAT activity did not differ among the placebo, atorvastatin 20 mg/day, and atorvastatin 80 mg/day treatment phases (22.7 ± 6.6 , 22.5 ± 6.1 , and 20.9 ± 5.9 nmol/ml, respectively; $P = 0.5$). Relative to placebo, only nonsignificant reductions in cholesteryl ester transfer protein activity were observed with atorvastatin 20 mg/day and 80 mg/day (37.3 ± 25.1 , 20.8 ± 24.2 , and 30.9 ± 11.5 nmol/ml, respectively; $P = 0.3$) as a result of large individual variations.

DISCUSSION

The main defect in the metabolism of apoB-containing lipoproteins observed in the mixed hyperlipidemia sub-

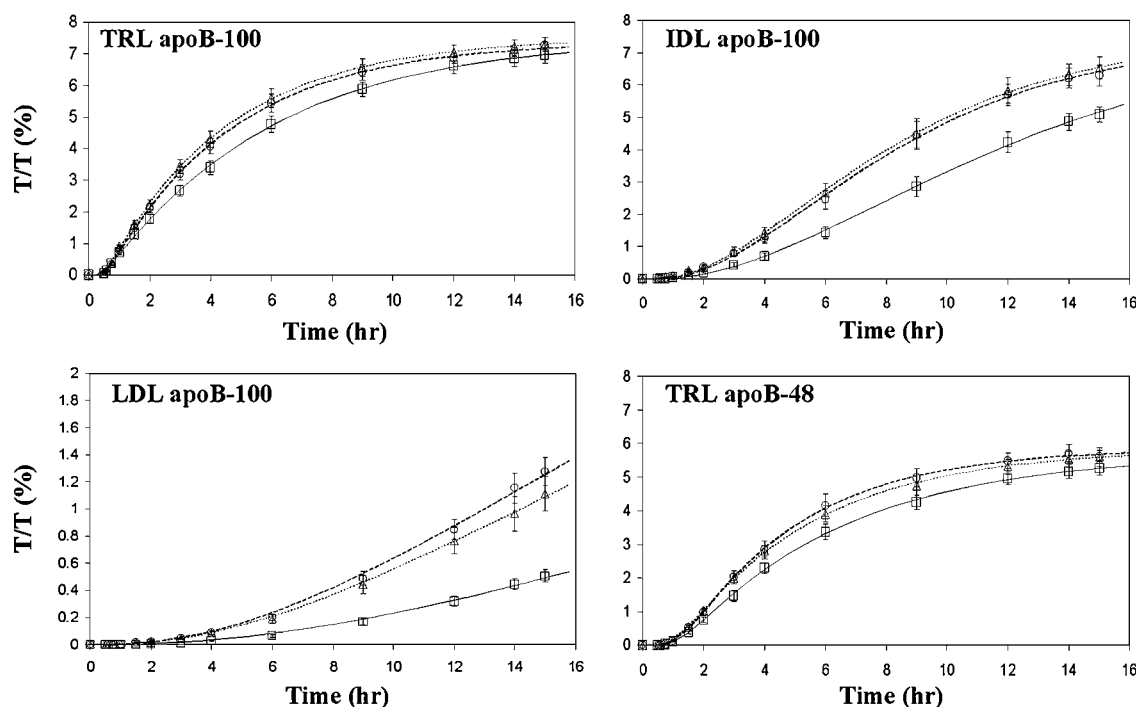


Fig. 1. Leucine tracer-tracee ratios (T/T; means \pm SD) of apolipoprotein B-100 (apoB-100) in triglyceride-rich lipoprotein (TRL), intermediate density lipoprotein (IDL), and LDL and of apoB-48 in TRL during the placebo (squares), atorvastatin 20 mg/day (triangles), and atorvastatin 80 mg/day (circles) phases. Lines represent the model-predicted values (placebo, continuous line; atorvastatin 20 mg/day, dotted line; atorvastatin 80 mg/day, dashed line).

TABLE 3. Kinetic parameters of apoB-100 in TRL, IDL, and LDL, of apoB-48 in TRL, and of apoA-I in HDL during the placebo and atorvastatin treatment phases

Parameter	Placebo	Atorvastatin, 20 mg/day	Atorvastatin, 80 mg/day	Percent Change ^a (P)	Percent Change ^b (P)	Percent Change ^c (P)
TRL apoB-100						
Pool size (mg)	369 ± 103	277 ± 67	307 ± 94	-22 (0.019)	-13 (0.129)	13 (0.275)
FCR (pools/day)	4.19 ± 1.45	5.94 ± 1.65	5.57 ± 1.33	47 (0.0003)	42 (0.011)	-2 (0.448)
PR (mg/kg/day)	17.7 ± 4.44	19.7 ± 6.08	19.9 ± 4.49	12 (0.206)	16 (0.165)	4 (0.840)
Conversion to IDL (mg/kg/day)	7.66 ± 3.05	8.33 ± 2.34	6.70 ± 2.30	21 (0.467)	45 (0.422)	41 (0.147)
Conversion to LDL (mg/kg/day)	3.53 ± 2.26	3.68 ± 1.68	4.50 ± 1.86	15 (0.686)	54 (0.205)	39 (0.202)
IDL apoB-100						
Pool size (mg)	121 ± 40	74 ± 20	77 ± 17	-35 (0.007)	-33 (0.003)	9 (0.797)
FCR (pools/day)	5.18 ± 1.46	8.11 ± 2.89	9.07 ± 3.82	72 (0.031)	77 (0.008)	18 (0.435)
PR (mg/kg/day)	7.66 ± 3.05	7.06 ± 2.10	7.97 ± 2.72	10 (0.633)	14 (0.726)	22 (0.442)
Conversion to LDL (mg/kg/day)	7.46 ± 2.87	7.00 ± 2.29	6.26 ± 2.72	-6 (0.387)	-8 (0.277)	-10 (0.113)
LDL apoB-100						
Pool size (mg)	3,682 ± 975	2,186 ± 674	1,908 ± 605	-41 (0.0001)	-48 (0.0001)	-12 (0.043)
FCR (pools/day)	0.25 ± 0.06	0.43 ± 0.11	0.530 ± 0.13	81 (0.0009)	115 (0.0001)	27 (0.044)
PR (mg/kg/day)	11.0 ± 2.41	10.9 ± 1.58	11.7 ± 2.48	6 (0.960)	8 (0.211)	8 (0.392)
TRL apoB-48						
Pool size (mg)	24 ± 11	17 ± 6	16 ± 5	-24 (0.054)	-31 (0.010)	-1 (0.421)
FCR (pools/day)	3.03 ± 0.78	3.35 ± 0.89	4.30 ± 2.75	11 (0.102)	37 (0.131)	24 (0.246)
PR (mg/kg/day)	0.87 ± 0.36	0.66 ± 0.23	0.77 ± 0.45	-16 (0.104)	-9 (0.470)	14 (0.317)
HDL apoA-I						
Pool size (mg)	3,880 ± 981	3,812 ± 729	3,649 ± 658	0 (0.671)	-3.8 (0.208)	-3.8 (0.161)
FCR (pools/day)	0.261 ± 0.040	0.255 ± 0.040	0.256 ± 0.029	-2.1 (0.496)	-1.1 (0.521)	1.5 (0.843)
PR (mg/kg/day)	12.10 ± 2.50	11.89 ± 2.78	11.21 ± 1.84	-1.3 (0.733)	-5.3 (0.186)	-3.1 (0.293)

FCR, fractional catabolic rate; PR, production rate. Values are means ± SD.

^a Percent change, atorvastatin 20 mg/day versus placebo.

^b Percent change, atorvastatin 80 mg/day versus placebo.

^c Percent change, atorvastatin 80 mg/day versus atorvastatin 20 mg/day.

jects participating in this study was a reduction in clearance, as the mean TRL, IDL, and LDL apoB-100 FCRs in these subjects were 4.2, 5.2, and 0.25 pools/day, respectively, compared with 6.6–7.1, 7.4–12.0, and 0.26–0.29 pools/day, respectively, in normolipidemic men and women studied previously in our laboratory (15). The apoB-100 PR values of our subjects were within the normal ranges of normolipidemic men and women (15).

Consistent with previous reports (6, 22, 23), we found that atorvastatin decreased the pool sizes of TRL, IDL, and LDL apoB-100 by increasing the apoB-100 FCRs in these fractions. Decreasing plasma apoB-100-containing lipoprotein levels is achieved by the same kinetic modification, namely an increase in clearance, at both moderate and high doses of atorvastatin. This outcome is likely mediated by the inhibitory effect of atorvastatin on

cholesterol synthesis and the ensuing activation of the cholesterol-sensing transcription factor sterol-regulatory element binding protein, which then mediates the activation of the expression of the LDL receptor, one of its target genes (24). Alternatively, atorvastatin may also induce changes in the composition of the apoB-containing lipoproteins and thus increase their affinity for the LDL receptor (25). In addition, atorvastatin has been shown to increase the expression and activity of lipoprotein lipase (26), thus favoring rapid lipolysis of lipoproteins. A novel finding in our study was the observation that increasing the dose of atorvastatin from 20 to 80 mg/day significantly enhanced LDL apoB-100 FCR, leading to a significant further reduction in plasma LDL-C levels.

Atorvastatin also caused an increase in the plasma levels of markers of intestinal cholesterol absorption, the plant

TABLE 4. Effect of 20 mg/day and 80 mg/day atorvastatin on markers of cholesterol synthesis and intestinal absorption

Marker	Placebo	Atorvastatin, 20 mg/day	Atorvastatin, 80 mg/day	Percent Change ^a	Percent Change ^b	Percent Change ^c
Lathosterol	123.7 ± 43.5	36.6 ± 13.7 ^d	26.7 ± 6.7 ^d	-69 ± 10	-76 ± 7	-18 ± 31
Campesterol	237.2 ± 55.2	356.3 ± 104.2 ^d	430.7 ± 120.4 ^d	48 ± 15	71 ± 41	11 ± 33
β-Sitosterol	92.9 ± 22.2	126.3 ± 34.2 ^d	172.6 ± 32.9 ^d	36 ± 16	79 ± 39	25 ± 37
Lathosterol-campesterol ratio	0.56 ± 0.25	0.11 ± 0.08 ^e	0.07 ± 0.03 ^{e,f}	-79 ± 6	-86 ± 3	-27 ± 13
Lathosterol-β-sitosterol ratio	1.42 ± 0.63	0.32 ± 0.20 ^e	0.16 ± 0.06 ^{e,f}	-77 ± 7	-87 ± 3	-37 ± 11

Nonratio values are reported as 10² μmol/mmol cholesterol.

^a Percent change, atorvastatin 20 mg/day versus placebo.

^b Percent change, atorvastatin 80 mg/day versus placebo.

^c Percent change, atorvastatin 80 mg/day versus atorvastatin 20 mg/day.

^d Significantly different from placebo, *P* < 0.005.

^e Significantly different from placebo, *P* < 0.001.

^f Significantly different from atorvastatin 20 mg/day, *P* < 0.02.

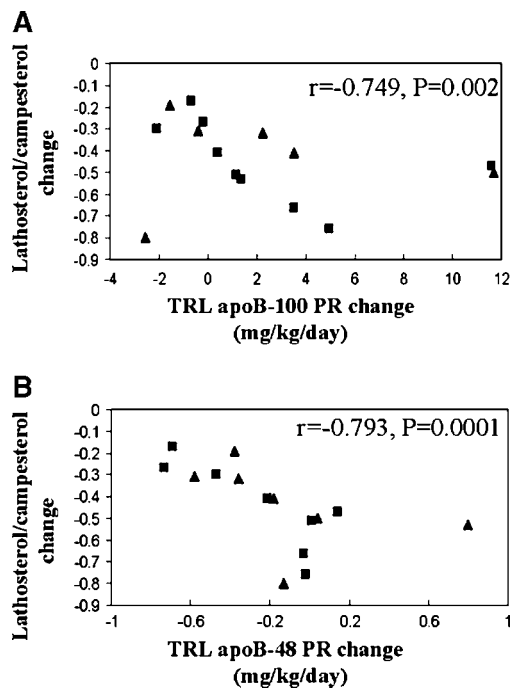


Fig. 2. A: Association between the change in plasma lathosterol-campesterol ratio and the change in TRL apoB-100 production rate (PR) during treatment with 20 mg/day (squares; $r = -0.786$, $P < 0.02$) and 80 mg/day (triangles; $r = -0.657$, $P = 0.15$) atorvastatin. B: Association between the change in plasma lathosterol-campesterol ratio and the change in TRL apoB-48 PR during treatment with 20 mg/day (squares; $r = -0.762$, $P < 0.03$) and 80 mg/day (triangles; $r = -0.857$, $P < 0.01$) atorvastatin.

sterols campesterol and β -sitosterol. Statins have been shown previously to increase plasma plant sterol levels (20, 27), with the most potent statins, such as atorvastatin, causing a greater increase than less potent statins, such as simvastatin (28). The lathosterol-campesterol ratio is used as an indicator of cholesterol homeostasis and has been

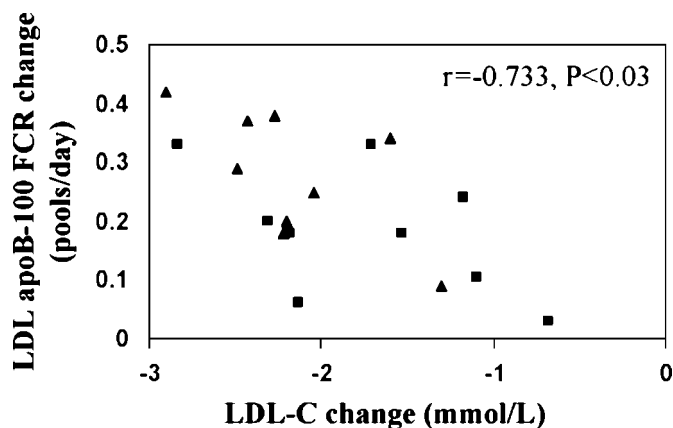


Fig. 3. Association between the change in LDL-cholesterol (-C) and the change in LDL apoB-100 fractional catabolic rate (FCR) during treatment with 20 mg/day atorvastatin (squares; $r = -0.583$, $P = 0.09$) and 80 mg/day atorvastatin (triangles; $r = -0.667$, $P < 0.05$).

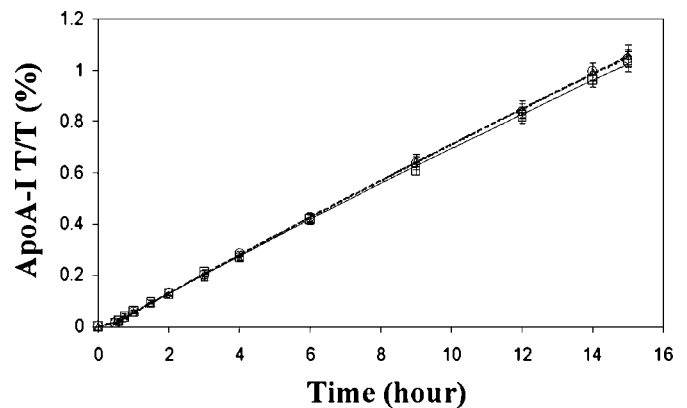


Fig. 4. Leucine tracer-tracee ratios (T/T; means \pm SD) of HDL apoA-I during the placebo (squares), atorvastatin 20 mg/day (triangles), and atorvastatin 80 mg/day (circles) phases. Lines represent the model-predicted values (placebo, continuous line; atorvastatin 20 mg/day, dotted line; atorvastatin 80 mg/day, dashed line).

shown to be positively associated with TRL apoB-100 pool size in normal and obese subjects (29). In our study, the lathosterol-campesterol ratio was decreased significantly during treatment with the higher dose of atorvastatin, compared with the lower dose. This was accounted for by both an increase in campesterol and a reduction in lathosterol levels. Moreover, we found that during treatment with atorvastatin, relative to placebo, the change in the lathosterol-campesterol ratio was significantly and inversely associated with the change in both TRL apoB-100 PR and TRL apoB-48 PR.

These findings indicate that individuals who experienced greater reductions in the lathosterol-campesterol ratio presented with larger increases in the production of apoB in liver and intestinal cells. We speculate that the change in cholesterol homeostasis associated with the use of more potent statins may increase the cholesterol pool destined for lipoprotein synthesis and therefore prevent a reduction in apoB production. Consistent with this concept is the observation that the majority of studies conducted with the lower potency lovastatin found that a reduction in TRL apoB-100 PR is involved in the decrease of TRL and LDL-C levels (2), whereas atorvastatin, one of the most potent statins, has been shown to predominantly affect apoB-100 FCR (6, 22, 23). However, differences in the characteristics of the subjects, study design, methodology, and kinetic model used may also account for some of the differences in results among the studies. The degree of change in lathosterol-campesterol ratio after treatment with atorvastatin may be subject to genetic variability, as it has been shown that the amount of LDL-C decrease with atorvastatin is influenced by genetic polymorphisms at the locus for ATP binding cassette G5/G8, a transporter involved in intestinal cholesterol absorption and biliary cholesterol excretion (30). In hypercholesterolemic subjects, a nonsignificant 18% reduction in TRL apoB-48 PR and a significant 21% increase in TRL apoB-100 PR have been observed with ezetimibe, a medication that inhibits intes-

tinal cholesterol absorption by blocking the Niemann-Pick C1 Like1 receptor (31). A direct comparison between the effects of ezetimibe and atorvastatin on cholesterol absorption and apoB kinetics is difficult because of the different targets of action of the two medications and the possible subsequent effects on cellular cholesterol synthesis and cellular distribution.

Plasma concentrations of apoB-48 were reduced significantly by both doses of atorvastatin. The reduction in plasma apoB-48 levels was mostly attributable to an increase in FCR, especially at the highest dose of atorvastatin; although these changes did not reach statistical significance, an increase in apoB-48 clearance was observed in seven of the nine subjects during the 80 mg/day atorvastatin regimen. We speculate that the reduction in apoB-48 levels during atorvastatin is mediated by an increased apoE-mediated uptake of chylomicrons by LDL receptors, whose increased expression also likely mediates the increased apoB-100 FCR. Although our study is the first to investigate the effects of a statin on the kinetics of apoB-48, previous studies using oral fat load tests have shown a reduction in both TG and apoB-48 in the chylomicron fraction during atorvastatin treatment, compared with placebo, in subjects with hypertriglyceridemia (32), type III hyperlipoproteinemia (33), or coronary heart disease (34). However, in subjects with familial combined hyperlipidemia, no effect of atorvastatin on chylomicron apoB-48 concentration was observed (35, 36). Studies with chylomicron-like particles containing labeled cholesterol and TG have indicated a significant decrease in cholesterol residence time during treatment with 10 mg/day and 40 mg/day atorvastatin in patients with coronary heart disease (37). Our study, and most previous studies, suggest that the decrease in chylomicrons accompanying atorvastatin treatment may be attributable to an accelerated clearance of apoB-48.

Previous kinetic studies of the effects of two other statins, pravastatin and lovastatin, on apoA-I metabolism have indicated an increase in apoA-I PR (38, 39). In our study, no significant effect of atorvastatin on apoA-I PR or FCR was observed. These results are in agreement with previously published studies indicating no effect of atorvastatin on the kinetics of apoA-I (6, 40–43). The change in HDL-C with atorvastatin was independent of apoA-I kinetics. The absence of significant changes in apoA-I concentrations and kinetic parameters notwithstanding, treatment with both the lower and higher doses of atorvastatin causes a significant change in the HDL subpopulation distribution, as reported previously (44). The change in HDL subpopulations suggests an effect of atorvastatin on HDL remodeling, possibly mediated via a reduction in plasma TG levels and the associated reduction in cholesteryl ester transfer protein activity (6, 45, 46). Some intervention trials have shown that the changes in plasma HDL-C levels during treatment with statins are significant predictors of coronary heart disease outcome even after controlling for the LDL-C-lowering effect (47, 48).

In conclusion, atorvastatin affects mostly the metabolism of apoB-containing lipoproteins through an increase in

their rate of catabolism. Increasing the dose of atorvastatin potentiates the LDL-C-lowering effect through further increases in LDL apoB-100 clearance. The atorvastatin-mediated change in cellular cholesterol homeostasis may participate in regulating apoB PR. **RR**

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