Lipid and Apolipoprotein Levels and Distribution in Patients With Hypertriglyceridemia: Effect of Triglyceride Reductions With Atorvastatin

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Atorvastatin is a new hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been demonstrated to be efficacious in reducing both triglyceride (TG) and cholesterol (CHOL) levels in humans. Twenty-seven (N = 27) patients with primary hypertriglyceridemia (TG > 350 mg/dL) were studied before and after 4 weeks on atorvastatin treatment at a dosage of either 20 (n = 16) or 80 (n = 11) mg/d. The present report examines changes in the plasma levels of several apolipoproteins, including apolipoprotein C-II (apoC-II), apoC-III, and apoE, after atorvastatin. Dose-dependent reductions in both CHOL (20.3% *v* 43.1%) and TG (26.5% *v* 45.8%) for the low and high dose, respectively, have been reported in these individuals. In addition to the reductions in apoB commonly associated with the use of HMG-CoA reductase inhibitors, significant reductions in apoE (37% and 49%), apoC-II (28% and 42%), and apoC-III (18% and 30%) were observed with this agent at the 20- and 80-mg/d dosage, respectively. Using fast protein liquid chromatography (FPLC) to fractionate whole plasma according to particle size, the effect of atorvastatin on lipid and apolipoprotein distribution in 20 lipoprotein fractions was also determined. Our results indicate that after 4 weeks on atorvastatin, (1) there was a 2-fold increase in the CHOL content as assessed by the CHOL/apoB ratio for 13 subfractions from very-low-density lipoprotein (VLDL) to small low-density lipoprotein (LDL); (2) there was a statistically significant reduction in the percentage of plasma apoB associated with VLDL-sized particles (30.5% *v* 26.8%); (3) there was a preferential reduction in plasma apoE from non-apoB-containing lipoproteins with treatment; (4) the losses of apoC-II and apoC-III, on the other hand, were comparable for all lipoprotein fractions; and (5) the fraction of plasma TG associated with HDL was increased after treatment. These changes in lipids and apolipoproteins did not depend on the dose of atorvastatin. There was, on the other hand, a dose-dependent reduction in cholesteryl ester transfer protein (CETP) activity, defined as the percentage of ^{3}H-cholesteryl oleate transferred from high-density lipoprotein (HDL) to LDL. CETP activity was reduced by 10.3% and 26.4% with the low and high dose of atorvastatin. Together, these composition data would be consistent with a net reduction in the number of TG-rich lipoproteins that may be explained by (1) a reduction in VLDL synthesis, (2) a preferential removal of VLDL without conversion to LDL, and (3) a preferential accelerated removal of a subpopulation of LDL.

HEPATIC hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) constitute a class of compounds that are efficient in reducing plasma cholesterol (CHOL).[1-3] The majority of the available statins, eg, lovastatin, pravastatin, simvastatin, and fluvastatin, have only a modest effect on plasma triglyceride ([TG] 12% to 22% reduction).[4,5] Only niacin and fibric acid derivatives (fibrates) have been available for the treatment of patients with significantly elevated plasma TG.[6,7] In a few studies of patients with combined hyperlipidemia (elevations in both TG and CHOL), combination therapies with one of the statins and either niacin or fibrates have been reported to be effective in decreasing both TG and CHOL.[8,9] Atorvastatin is a new synthetic statin that has been demonstrated to be safe and efficacious in reducing plasma low-density lipoprotein cholesterol (LDL-CHOL) and TG both in patients with hypercholesterolemia[10,11] and in patients with primary hypertriglyceridemia.[11] The basis of this TG reduction with an inhibitor of HMG-CoA reductase is not known.

In view of the significant reductions in TG (27% to 46%) observed with atorvastatin,[11] one would expect the plasma levels of apolipoproteins such as apolipoprotein C-II (apoC-II), apoC-III, and apoE to be affected since they are known to modulate the metabolism of TG. ApoC-III, which is present at high concentrations in patients with hypertriglyceridemia, has been shown to have an inhibitory effect on the activity of lipoprotein lipase,[12,13] as well as the rate of uptake of very-low-density lipoprotein (VLDL) remnants by the liver.[14,15] Reductions in the concentration of this protein are associated with lower concentrations of VLDL-TG and VLDL-apoB. Enrichment of apoB-containing lipoproteins with apoE, on the other hand, has been demonstrated to result in accelerated uptake of these particles via the remnant receptor[15-17] with a subsequent reduction in the interconversion of apoB from VLDL to LDL.[18] When patients with primary hypercholesterolemia are treated with HMG-CoA reductase inhibitors, changes in the concentration of plasma apolipoproteins other than apoB are minimal.[19-21] The effect of atorvastatin on the plasma concentration of apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE in patients with primary hypertriglyceridemia will be presented in this report. This is the first study of the effect of a HMG-CoA reductase inhibitor on lipid and apolipoprotein composition in patients with elevated fasting TG.

In contrast to apoB and apoA-I, which remain associated with a single particle throughout their life span in plasma, other apolipoproteins such as apoE, apoC-II, and apoC-III are distributed among several lipoprotein classes, and changes in the plasma levels alone may not be sufficient to explain the impact of atorvastatin therapy on the metabolism of plasma lipoproteins. We have used fast protein liquid chromatography (FPLC) to fractionate whole plasma into 20 subfractions of lipoproteins

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Submitted September 2, 1998; accepted August 29, 1999.

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0026-0495/00/4902-0017$10.00/0

ranging from VLDL to high-density lipoprotein (HDL),[22] and the effect of atorvastatin on the lipid and apolipoprotein composition of these subfractions will be presented.

SUBJECTS AND METHODS

Subjects and Study Design

Samples for the present analysis were obtained from a subset of hypertriglyceridemic individuals participating in a double-blind, parallel-group randomized trial with atorvastatin. The details of the trial and the effects of atorvastatin on primary lipid parameters have been previously published.[11] Only plasma samples from the 27 patients who completed the 4-week treatment period with either 20 mg/d (n = 16) or 80 mg/d (n = 11) atorvastatin were available for the present study. In all subjects, baseline measurements were determined in samples collected at the visit prior to randomization, and posttreatment measurements were obtained from samples collected 4 weeks later.

FPLC Procedure

The validation of the FPLC procedure is presented in detail elsewhere.[22] In brief, the FPLC system (Pharmacia Biotech, Piscataway, NJ) consisted of a single Superose 6HR 10/30 column equilibrated in phosphate-buffered saline (0.15 mol/L NaCl, 0.05 mol/L PO_4^-, 0.01% EDTA, and 0.02% NaN_3, pH 7.4). A multisample injector adapted with a 200-μL sample loop was available for sample loading, and the flow rate was maintained constant at 0.3 mL/min with the collection of 20 fractions (0.6-mL vol). The FPLC was programed to include a 40-minute wash after each sample injection. As part of the routine procedure, severely hypertriglyceridemic samples (TG > 750 mg/dL) were diluted in saline (0.15 mol/L NaCl and 0.01% EDTA) prior to application onto the FPLC column. Lipid concentrations in the applied samples were always less than 300 mg/dL for CHOL and less than 750 mg/dL for TG.

The eluted fractions collected in cryovials were assayed for TG and CHOL by enzymatic methods using the sensitive user-defined channels on the CX-5 autoanalyzer (Beckman Instruments, Fullerton, CA), which are calibrated with standards of 35 and 25 mg/dL for TG and CHOL, respectively. These sensitive channels have a linear range of 0.4 to 50 mg/dL and a coefficient of variation (CV) less than 3% for both TG and CHOL.[22] The fractions were tightly capped and stored at 4°C for the determination of apolipoprotein levels by enzyme-linked immunosorbent assay ([ELISA] approximately 1 to 2 weeks later).

Determination of Apolipoprotein Levels by ELISA

Apolipoprotein concentrations were determined in each eluted fraction either by sandwich ELISA (apoA-I, apoA-II, apoB, and apoE) or by competitive ELISA (apoC-II and apoC-III) using specific immunoglobulin G isolated by immunoaffinity chromatography from polyclonal antisera available in-house. The characteristics of these antisera have been previously reported by our group using radioimmunoassay.[23]

The linear range for the apoA-I and apoB assays is 3 to 30 ng/mL with an interassay CV of 4.7% and 6.6% for apoA-I and apoB, respectively. The intraassay CV for apoA-I and apoB is 2.8% and 3%, respectively. The linear range for the apoA-II assay is 1 to 7.5 ng/mL with an interassay and intraassay CV of less than 8.8% and 1.9%, respectively. The linear range for the apoE assay is 0 to 9.6 ng/mL with an interassay and intraassay CV of 6.6% and 4.4%, respectively. The logarithmic-linear range for the apoC-II assay is 1.3 to 54 ng/mL with an interassay and intraassay CV of 5.7% and 4.0%, respectively.[24] The log-linear range for the apoC-III assay is 14 to 123 ng/mL with an interassay and intraassay CV of 7.9% and 6.7%, respectively.[24] With the exception of the apoB assay that was calibrated with whole LDL, all other assays were calibrated with purified apolipoproteins. The presence of high TG concentrations in the form of Intralipid (Upjohn, Kalamazoo, MI) has been shown not to affect the determination of apoC-II and apoC-III by ELISA.[24]

Determination of LDL Particle Size by Nondenaturing Gradient Gel Electrophoresis

LDL particle size was determined using a nondenaturing segmental gradient gel previously reported by our group.[25] Plasma samples were prestained for 2 hours at room temperature with 0.6% Sudan black in ethylene glycol (3:1) prior to loading onto the gel. Pretreatment and posttreatment samples were analyzed in adjacent lanes, and the position of LDL bands was determined using the LKB laser densitometer (LKB Pharmacia, Piscataway, NJ). The interassay and intraassay CVs for LDL particle diameter are 1.16% and 0.47%.[25]

Determination of Maximal CETP Activity

Isotopic assays for CETP activity were performed for all participants using a common source of HDL_3 and LDL as donor and acceptor, respectively.[26,27] LDL (d 1.020 to 1.063 g/mL), HDL_3 (d 1.15 to 1.21 g/mL), and the fraction at d > 1.21 g/mL were isolated from pooled fasting plasma obtained from a group of young healthy normolipidemic controls by sequential ultracentrifugation. The LDL and HDL_3 fractions were subjected to recentrifugation at the appropriate density to remove potential contaminants. HDL_3 was labeled in vitro with ^{3}H-cholesteryl oleate by incubation with d > 1.21 g/mL as a source of LCAT.[28]

For the determination of CETP activity, 20 μL of a 1:3 dilution (in saline) of the patient's plasma was added to a mixture containing 50 μL ^{3}H-HDL (40 mg/dL HDL-CHOL) and 200 μL LDL (200 mg/dL LDL-CHOL). Incubations were performed in triplicate at 37°C for 24 hours, and the tubes were quickly chilled by immersion in crushed ice. LDL was precipitated by the addition of 27 μL heparin manganese solution (0.3 mg/mL heparin and 0.92 mol/L $MnCl_2$) as described by Tato et al,[26,27] and radioactivity remaining in the HDL supernate was determined.

Data Analysis

VLDL was defined to include fractions 4 through 8 (elution vol, 6.6 to 9.0 mL inclusive), LDL included fractions 9 through 16 (elution vol, 9.6 to 14.4 mL inclusive), and HDL included fractions 17 through 23 (elution vol, 15 to 19.8 mL inclusive). For the present analysis, lipid and apolipoprotein contents in each lipoprotein class were obtained by summing the masses in all of the fractions corresponding to that lipoprotein class.[22] Paired Student's *t* test was used to assess the significance of the intervention with atorvastatin using the SigmaStat program (Jandel Scientific, San Rafael, CA).

RESULTS

Effect of Storage on Lipoprotein Composition Assessed by FPLC

Figure 1 illustrates the similarity in the CHOL and TG content of VLDL obtained by ultracentrifugation using fresh plasma and VLDL obtained by FPLC using plasma aliquots that have been frozen for 11 to 13 months. The slope of the linear regression for the 2 estimates of VLDL-CHOL (Fig 1A) was 1.13 with an intercept of −2.08 (r^2 = .92). The mean difference (mean ± SD) in VLDL-CHOL was −9.20 ± 23.86 mg/dL, or 3.9% (Fig 1B). The slope of the linear regression between the 2 estimates of VLDL-TG (Fig 2C) was 1.08 with an intercept of 31.05 (r^2 = .98). The mean difference in VLDL-TG was −62.01 ± 46.76 mg/dL, or 12.8%. The mean ratio of the 2 estimates of LDL-CHOL (FPLC *v* ultracentrifuge) was 1.02 ± 0.116, and of HDL-CHOL (FPLC *v* dextran sulfate precipitation) 1.10 ± 0.218.[22] It should be noted that the lipid values and composition data from the ultracentrifuge used for the purpose

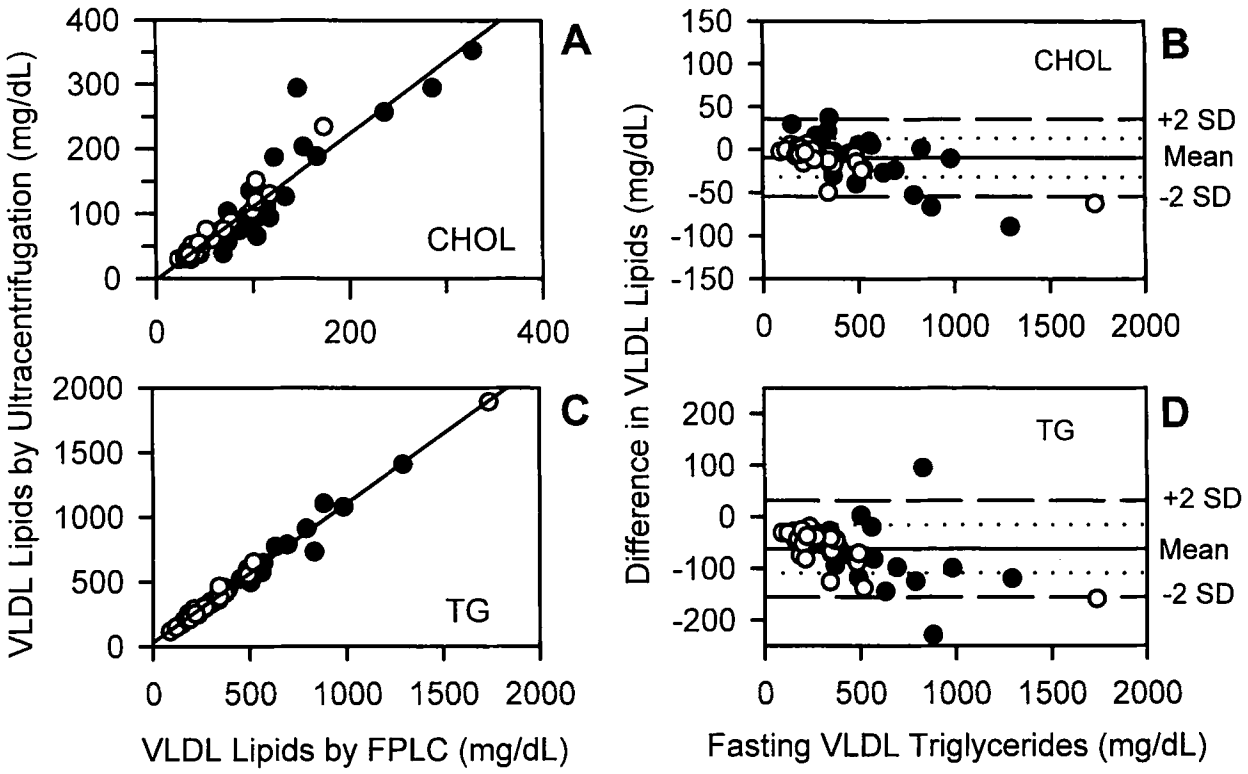

Fig 1. Comparison between FPLC and ultracentrifuge for VLDL-CHOL and VLDL-TG. Composition of VLDL isolated by ultracentrifugation was available from the Core Laboratory for Clinical Studies using freshly collected plasma samples. Fractionation of whole plasma by FPLC was performed after 12-13 months of storage at −80°C. (●) Baseline samples; (○) posttreatment samples. (A) Linear correlation between the 2 estimates for VLDL-CHOL. (B) Differences in VLDL-CHOL between the 2 estimates as a function of fasting TG. (—) Mean value (3.4%), (····) ±1 SD interval, (---) ±2 SD interval. (C) Linear correlation between the 2 estimates for VLDL-TG. (D) Differences in VLDL-TG between the 2 methods presented as a function of fasting plasma TG. The mean percent difference was 12.8% for plasma samples with TG from 130 to 1,850 mg/dL.

of this comparison were originally determined at a different laboratory using different enzymatic assays.

In this study, all posttreatment plasma samples were kept frozen for at least 12 months before FPLC analysis. For each participant, the pretreatment plasma sample was stored at −80°C for exactly 4 weeks more than the posttreatment sample. To examine the effect of storage on apolipoprotein distribution, we compared the amount of apoE and apoC-III in VLDL in a group of subjects with TG levels from 42 to 420 mg/dL recruited at Emory University. For this experiment, fresh plasma was fractionated by FPLC within 4 hours of blood collection, and an aliquot was kept at 4°C for 24 hours and then stored in a −80°C freezer. After exactly 4 weeks of storage, the stored samples were allowed to thaw slowly at 4°C overnight and were fractionated by FPLC. The apoE and apoC-III levels in VLDL were obtained by summing the respective concentrations in the eluted fractions corresponding to VLDL, and a ratio was calculated. Figure 2 presents the ratio of apoC-III and apoE in VLDL (fresh/frozen) as a function of TG levels. VLDL apolipoprotein levels were slightly higher in the fresh plasma compared with frozen plasma samples for both apoC-III and apoE, with a mean ratio of 1.13 ± 0.21 for apoC-III and 1.12 ± 0.15 for apoE. This was not statistically significant and, more importantly, the difference between fresh and frozen plasma did not appear dependent on plasma TG levels in this group of individuals.

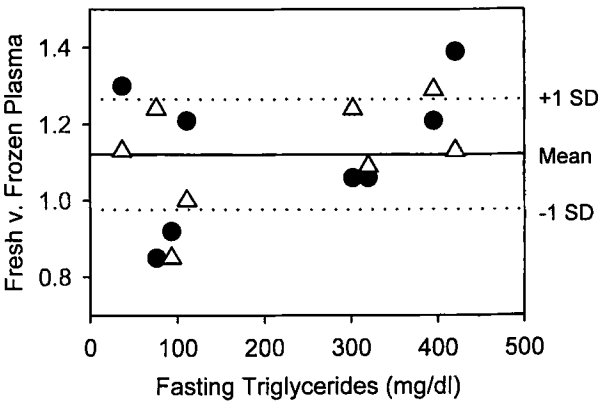

Fig 2. Effect of storage on apolipoprotein distribution. The ratio of apoE content in VLDL (△) obtained in freshly collected plasma to apoE content in VLDL obtained in plasma stored at −80°C for 1 month was not different in normotriglyceridemic and hypertriglyceridemic subjects. This ratio was also similar to the ratio of the VLDL-apoC-III content (●) between fresh and frozen samples. Dotted lines represent the ±1 SD interval around the mean; samples outside this interval are not limited to hypertriglyceridemic plasma.

Changes in Plasma Apolipoproteins

Table 1 presents the mean plasma lipid and apolipoprotein levels for the 2 groups at baseline and after 4 weeks of treatment

Table 1. Changes in Plasma Parameters (mean ± SD) With Atorvastatin

Parameter	Baseline	20 mg/d	% Change	Baseline	80 mg/d	% Change
CHOL	274 ± 49.5	186 ± 49.4*	−31.98 ± 14.7	247 ± 199.0	149 ± 121.8†	−38.73 ± 18.7
TG	670 ± 403.1	463 ± 432.8‡	−26.85 ± 35.6	623 ± 296.1	320 ± 136.9†	−36.71 ± 41.3
ApoA-I	139.0 ± 26.1	146.6 ± 30.7	+5.42 ± 10.5	109.4 ± 15.3	111.4 ± 18.6	+1.83 ± 12.5
ApoA-II	32.4 ± 6.56	33.2 ± 5.99	+3.72 ± 13.2	32.4 ± 5.85	32.8 ± 5.67	+1.24 ± 7.5
ApoB	118.4 ± 23.8	80.6 ± 18.7*	−31.15 ± 12.8	101.9 ± 35.1	65.9 ± 33.4*	−37.52 ± 15.9
ApoE	7.41 ± 4.95	4.68 ± 3.73*	−37.60 ± 18.2	5.30 ± 3.23	2.70 ± 1.93‡	−40.71 ± 38.6
ApoC-II	7.02 ± 1.71	5.07 ± 1.83*	−28.02 ± 15.4	7.32 ± 2.26	4.22 ± 1.19†	−38.45 ± 22.6
ApoC-III	24.04 ± 6.30	19.69 ± 6.65‡	−16.20 ± 20.3	27.12 ± 9.65	19.00 ± 6.84‡	−23.39 ± 34.2

NOTE. Statistical significance was tested by 2-tailed paired *t* test.
*$P < .0001$.
†$P < .005$.
‡$P < .02$.
$P > .05$ for all other parameters.

with either 20 mg/d (n = 16) or 80 mg/d (n = 11) atorvastatin. Statistically significant reductions in all apolipoproteins except apoA-I and apoA-II were demonstrated in both groups independently of the dose used. Small increases in apoA-I of 5.4% at 20 mg/d and 1.2% at 80 mg/d were noted, but these changes were not statistically significant.

Table 2 presents the Pearson correlation coefficients between the percent changes in the different parameters, including lipids, apolipoproteins, and LDL particle diameter. For this analysis, data from all patients were included independently of the dose used.

Changes in Lipid Distribution

Figure 3 illustrates changes in the distribution of CHOL, TG, apoB, and apoE as assessed by FPLC for 1 representative individual after 4 weeks on 80 mg/d atorvastatin (Fig 3A to D). The pattern of the changes was not different for the 2 doses of atorvastatin, and all subjects were combined for the subsequent composition analyses.

To assess the effect of atorvastatin on changes in the distribution of TG and CHOL among the lipoprotein fractions for all subjects, the percent distribution of each lipid was calculated as the ratio of the mass associated with each FPLC-defined lipoprotein fraction to the total mass summed over all 20 fractions. Figure 4 illustrates the changes in the percent of CHOL and TG in isolated lipoprotein fractions including VLDL, LDL, and HDL for all participants. Data from all subjects are included in this figure, since there was no difference in the pattern of changes with the low and high dose of atorvastatin. Tables 3 and 4 present the actual mean values for all measured parameters in terms of both percent of total plasma (Table 3) and concentration (Table 4). The mean percent of CHOL recovered in VLDL-sized fractions was reduced from 40.6% ± 8.99% to 32.0% ± 10.02% with atorvastatin ($P < .0001$). Despite a significant reduction (32% to 39%) in total CHOL with atorvastatin, the mean percent of total plasma CHOL associated with LDL was unchanged (46.07% ± 8.15% *v* 46.4% ± 7.95%; Fig 4B). On the other hand, the mean percent of CHOL in HDL was actually increased by atorvastatin from 13.6% ± 3.00% to 21.7% ± 5.74% ($P < .0001$). In comparing the pretreatment and posttreatment distribution, only the percent of CHOL recovered in HDL after treatment was correlated with the percent of CHOL in HDL at baseline ($R^2 = .62$; Fig 4C).

Despite a 27% to 37% reduction in total plasma TG with atorvastatin, the reduction in the percent of plasma TG associated with VLDL was modest, 76.4% versus 73.2% ($P < .02$; Fig 4D). There was no difference in the percent of total TG associated with LDL (Fig 4E and Table 3). There was a statistically significant increase in the percent of TG associated with HDL after atorvastatin therapy (median, 7.0% *v* 8.0%, $P < .0001$), and the percent of TG associated with HDL after treatment was correlated with the percent observed at baseline ($R^2 = .57$; Fig 4F).

Table 3 also presents the recovery data for CHOL, TG, apoA-I, apoA-II, apoB, apoE, apoC-II, and apoC-III obtained by summing the concentrations in the eluted fractions as compared with the total concentrations determined in whole plasma. There was no difference in the recoveries between baseline and posttreatment samples.

Table 4 presents the mean levels of CHOL, TG, apoB, apoE, apoC-II, and apoC-III in each lipoprotein fraction as defined by

Table 2. Pearson Coefficients for Percent Changes in Lipids and Apolipoproteins

Parameter	ApoA-II	ApoB	ApoC-II	ApoC-III	ApoE	CHOL	TG	LDL Size
ApoA-I	.670 (.0002)	.525 (.005)	.45 (NS)	.45 (NS)	.44 (NS)	.33 (NS)	.35 (NS)	−.144 (NS)
ApoA-II		.391 (NS)	.496 (NS)	.456 (NS)	.428 (NS)	.2 (NS)	.333 (NS)	.022 (NS)
ApoB			.446 (NS)	.362 (NS)	.483 (NS)	.198 (NS)	.250 (NS)	−.291 (.003)
ApoC-II				.864 (10^{-8})	.855 (10^{-8})	.167 (NS)	.844 (10^{-7})	−.549 (.005)
ApoC-III					.859 (10^{-7})	.163 (NS)	.851 (10^{-8})	−.533 (.005)
ApoE						.240 (NS)	.832 (10^{-7})	−.548 (.003)
CHOL							.226 (NS)	.046 (NS)
TG								−.493 (.009)

NOTE. P values ≥.01 are indicated as NS (not statistically significant).

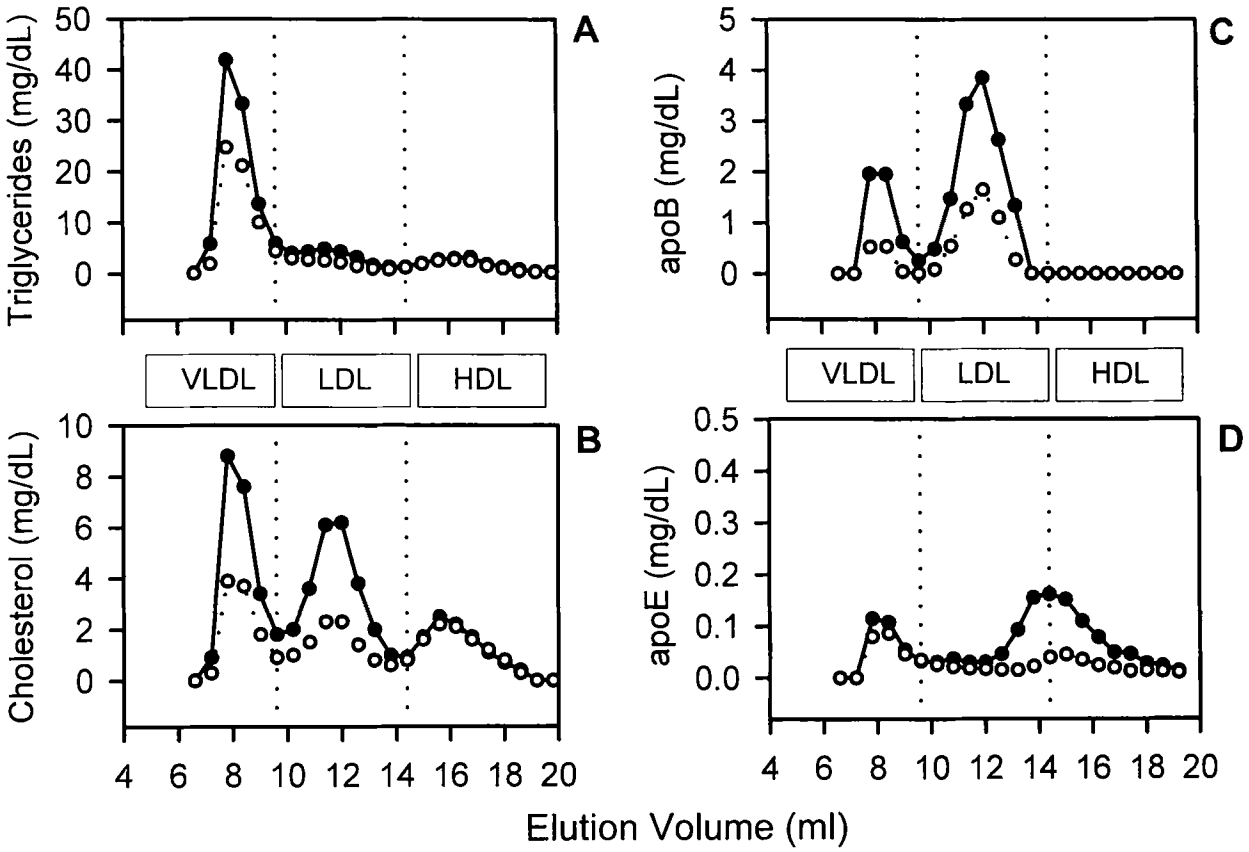

Fig 3. Effect of atorvastatin on the distribution of TG, CHOL, apoB, and apoE for a representative subject treated with atorvastatin (80 mg/d). Plasma levels of TG, CHOL, apoB, and apoE were 557 *v* 206, 231 *v* 119, 79*v* 59, and 3.4 *v* 1.3 for baseline *v* atorvastatin therapy. (●) Baseline sample, (○) posttreatment sample, (····) separation between VLDL, LDL, and HDL. VLDL corresponds to an elution vol of 6.8-9.6 mL, LDL has an elution vol of 10.2-14.4 mL, and HDL has an elution vol of 15-19.6 mL. (A) Reductions in TG can be demonstrated for VLDL and LDL with unchanged concentrations of TG in HDL. (B) Significant reductions in CHOL can be demonstrated in all fractions throughout the size range corresponding to apoB-containing lipoproteins. (C) Significant reductions in apoB could also be demonstrated in all fractions corresponding to apoB-containing lipoproteins. (D) With treatment, there was a preferential loss of apoE from HDL fractions, while apoE levels in VLDL-sized fractions appear to be minimally affected despite the reduction in VLDL-TG.

the FPLC. For this table, the individual percent distribution was multiplied by the plasma concentration for each subject and the mean ± SD was calculated for all 27 subjects. The statistical significance of the changes following treatment with atorvastatin was assessed by paired Student's *t* test. The reductions were significant for all fractions except HDL-CHOL. Of interest is the low *P* value for the changes in VLDL-apoE with treatment. In fact, using the 2-tailed *t* test, statistical significance

Table 3. Effect of Atorvastatin on the Distribution of Lipids and Apolipoproteins Among Lipoprotein Classes as Defined by FPLC (mean ± SD)

	Recovery (%)		Percent Distribution					
			VLDL		LDL		HDL	
Parameter	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CHOL	93.4 ± 3.25	95.1 ± 4.35	40.6 ± 8.99	32.0 ± 10.02*	46.1 ± 8.15	46.4 ± 7.95	13.6 ± 3.00	21.7 ± 5.74*
TG	89.5 ± 15.75	93.6 ± 7.25	76.4 ± 5.20	73.2 ± 6.96†	17.0 ± 4.25	17.9 ± 4.56	7.0 ± 5.3-8.0	8.0 ± 7.0-10.0*
ApoA-I	94.8 ± 9.41	99.5 ± 5.81	NA	NA	NA	NA	NA	NA
ApoA-II	100.5 ± 8.33	101.0 ± 5.63	NA	NA	NA	NA	NA	NA
ApoB	80.2 ± 6.85	83.1 ± 10.05	30.5 ± 9.37	26.8 ± 12.16†	69.5 ± 9.37	73.2 ± 12.16*	NA	NA
ApoE	76.8 ± 11.18	76.8 ± 9.03	20.3 ± 9.17	28.4 ± 14.42*	28.89 ± 7.48	27.0 ± 7.49	50.8 ± 12.23	44.6 ± 17.32*
ApoC-II	87.3 ± 8.73	86.8 ± 10.67	42.0 ± 13.99	40.6 ± 16.21	17.2 ± 6.45	16.72 ± 7.12	40.82 ± 12.3	42.7 ± 14.68
ApoC-III	75.0 ± 11.47	73.0 ± 12.63	22.5 ± 10.22	22.3 ± 12.62	27.5 ± 8.52	25.1 ± 8.97	50.0 ± 12.89	52.6 ± 14.08

NOTE. Since HDL-TG did not meet the normality test, the median (25th and 75th percentile) is presented for this parameter.
Abbreviation: NA, not available.
*$P < .0001$.
†$P < .01$.

Table 4. Effect of Atorvastatin on Lipoprotein Lipids and Apolipoproteins (mg/dL) as Defined by FPLC (mean ± SD)

	VLDL		LDL		HDL	
Parameter	Pre	Post	Pre	Post	Pre	Post
CHOL	119 ± 46	57 ± 26*	121 ± 23	78 ± 19*	35 ± 5	35 ± 5‖
TG	496 ± 207	309 ± 159*	109 ± 47*	65 ± 10*	43 ± 20	31 ± 9*
ApoB	34.74 ± 11.36	20.53 ± 9.18*	76.91 ± 18.73	54.08 ± 16.61*	NA	NA
ApoE	1.11 ± 0.38	0.90 ± 0.39§	1.78 ± 0.75	0.99 ± 0.52†	3.66 ± 2.35	1.98 ± 1.52†
ApoC-II	3.06 ± 1.16	1.89 ± 0.73*	1.18 ± 0.32	0.77 ± 0.26*	2.90 ± 0.84	2.05 ± 0.72*
ApoC-III	5.86 ± 2.79	4.06 ± 1.68†	6.97 ± 2.49	5.10 ± 2.02†	12.46 ± 3.59	10.61 ± 3.82‡

*$P < .0001$ for 2-tailed Student's t test.
†$P < .001$ for 2-tailed Student's t test.
‡$P < .01$ for 2-tailed Student's t test.
§$P < .05$ for 1-tailed Student's t test; not significant for 2-tailed Student's t test.
‖Not significant.

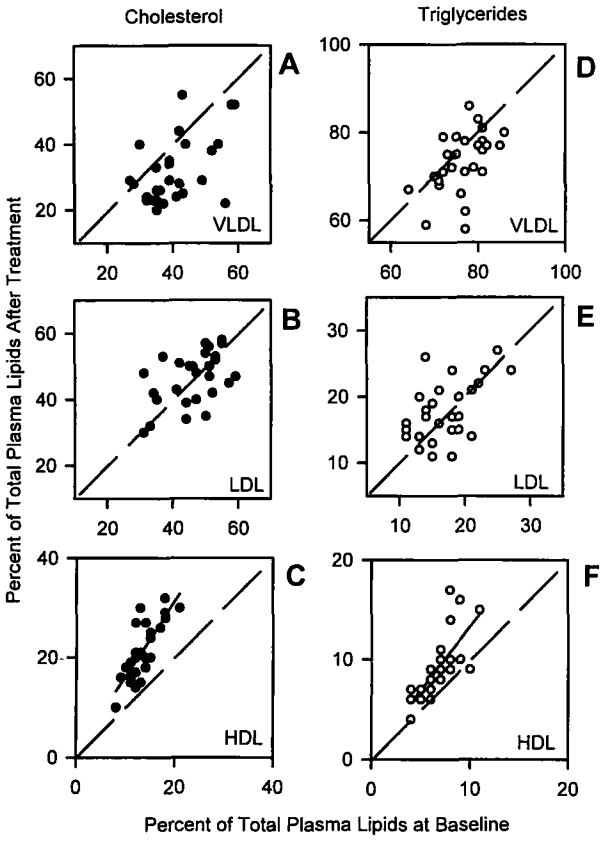

Fig 4. Changes in the distribution of plasma lipids among the 3 major classes of lipoproteins as defined by FPLC. (●) CHOL, (○) TG, (---) line of identity (slope of 1) between baseline and posttreatment samples. (A) The majority of data points are below the identity line, demonstrating an overall reduction in the percent of CHOL in VLDL after treatment. (B) Data points are scattered on both sides of the identity line, suggesting a lack of change in the percent of CHOL associated with LDL after therapy. (C) In the case of HDL, all data points were above the line of identity, demonstrating a significant increase in the percent of CHOL in HDL for all subjects ($r^2 = .62$). (D) There was a modest reduction in the percent of TG associated with VLDL, as demonstrated by the majority of data points being below the line of identity. (E) There was no change in the percent of plasma TG recovered in the LDL fraction. (F) For HDL, with the exception of 3 points, all points are above the line of identity, suggesting an increase in the percent of plasma TG in HDL ($r^2 = .57$).

could be demonstrated for all parameters except VLDL-apoE distribution; the 1-tailed test was used to demonstrate the weak statistical significance for this parameter.

Changes in the Composition of ApoB-Containing Lipoproteins

With a 31% to 37% reduction in total apoB with atorvastatin, there was a shift of apoB from VLDL-sized particles to LDL-sized particles (Table 3). There was no difference in the distribution for the 2 doses assessed, and the data from both dose levels were included for all subsequent analyses. The mean percent of plasma apoB recovered in the range of LDL-sized particles increased from 69.5% to 73.2% after atorvastatin ($P < .0001$). For each fraction eluted from the FPLC, we were able to determine the ratio of CHOL to apoB and the ratio of TG to apoB before and after the intervention. Figure 5A illustrates the change in the CHOL:apoB ratio for 10 lipoprotein fractions spanning the VLDL and LDL size range. In a preliminary analysis, no difference was found between the low and high doses with respect to the change in lipoprotein composition. For each fraction, the mean ratio for all 27 subjects (11 treated with high dose and 16 with low dose) is presented. To examine the trend across all particle sizes, data from the 7 fractions corresponding to IDL + LDL were used to determine the linear regression between the composition before and after treatment. With the concomitant reductions in CHOL and apoB with atorvastatin, the remaining apoB-containing particles had more CHOL per particle. On average, there was a 2-fold increase in CHOL content per particle. With respect to the TG:apoB ratio (Fig 5B), there was only a modest increase (13%) after atorvastatin therapy for all apoB-containing fractions, and it was not statistically significant.

In the present study of patients with hypertriglyceridemia, this increase in the lipid:apoB ratio occurred over a wide range of lipoprotein subfractions and did not result in a statistically significant increase in the particle diameter of the major LDL peak as assessed by gradient gel electrophoresis. The mean LDL particle diameter was 24.6 nm at baseline, as compared with 24.8 nm after the high dose (NS). In the low-dose group, the mean LDL particle diameter increased from 24.1 nm at baseline to 24.4 nm after treatment (NS, data not shown). The present technology does not allow a shift in particle size distribution to be assessed.

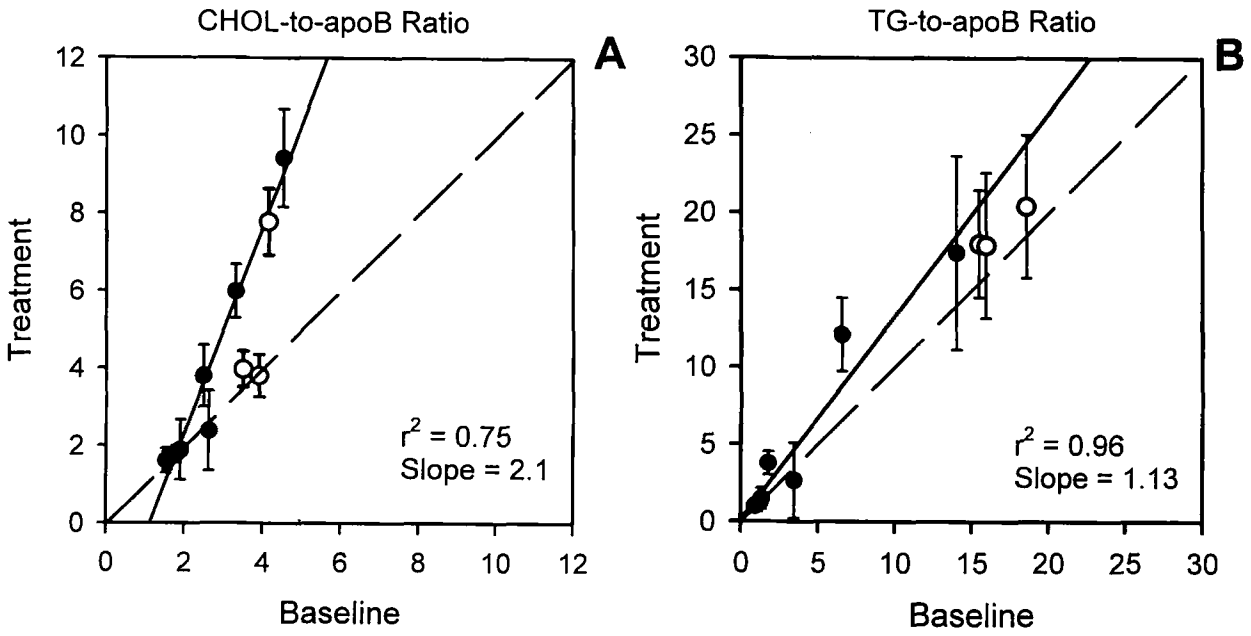

Fig 5. Changes in the composition of subclasses of apoB-containing lipoproteins. Each point represents the mean composition calculated for all 27 subjects independently of dose. (○) Fractions corresponding to VLDL-sized particles, (●) LDL-sized particles, (---) line of identity if there was no change in the lipid contents of apoB-containing particles after treatment with atorvastatin, (—) linear regression line through all 7 solid points corresponding to LDL-sized particles. (A) CHOL to apoB ratio in eluted FPLC fractions across the spectrum of apoB-containing lipoproteins. On average, there was a 2-fold increase in CHOL content per particle with a net reduction in the number of particles as assessed by apoB levels. (B) TG to apoB ratio in each fraction across the entire spectrum of apoB-containing lipoproteins. There was a modest increase in TG content per particle (13%), but this was not statistically significant.

Changes in ApoE Distribution With Atorvastatin

With the reduction in total plasma apoE after atorvastatin treatment, there were reductions in apoE mass associated with all 3 major lipoprotein classes (Table 4). Of special interest, of the 3 fractions isolated by FPLC, the reduction in apoE content was the least (1.11 *v* 0.90 mg/dL) for VLDL and the greatest for HDL (3.66 *v* 1.98 mg/dL). This point is illustrated in Fig 6, which presents the percent of total apoE associated with VLDL-sized particles (fractions 4 to 8), LDL-sized particles (fractions 9 to 16), and HDL-sized particles (fractions 17 to 23) at baseline and after atorvastatin therapy. There was a statistically significant increase in the percent of apoE associated with VLDL-sized particles after treatment from a mean of 20.3% ± 9.17% to 28.4% ± 14.42% ($P < .0001$). For this analysis, all regression lines are assumed to pass through the origin. The slope of the linear regression between the baseline and posttreatment percent distribution was 1.38 ($R^2 = .76$), or a mean increase of 38%, with atorvastatin. There was a concomitant decrease in the percent of plasma apoE associated with HDL from a median of 48.9% (25th to 75th percentile, 39.6% and 60.6%) to 46.0% (27.7% and 62.5% for the 25th to 75th percentile, respectively). The slope of the linear regression was 0.898 for HDL-apoE before and after treatment, with an intercept almost identical to 0. There was no statistical difference in the percent of apoE in LDL before and after treatment, 28.9% ± 7.48% versus 27.0% ± 7.49%. The slope of the linear regression for the percent of apoE in LDL was 0.92.

This preferential change in apolipoprotein distribution after atorvastatin therapy appeared to be specific for apoE and could not be demonstrated for the other 2 exchangeable apolipoproteins, namely apoC-III (Table 3 and Fig 7) and apoC-II (Table 3). There was a statistically significant reduction in the concentration of apoC-II and apoC-III in all 3 lipoprotein fractions as isolated by FPLC (Table 4).

Changes in CETP Activity With Atorvastatin

Figure 8 illustrates the changes in CETP activity following atorvastatin therapy. Three subjects in the low-dose group were not included in this analysis due to insufficient plasma volume. The mean percent of CE transferred from HDL to LDL was reduced by 10.3%, from 32.7% ± 2.84% to 29.3% ± 2.95%, with the low dose ($P < .001$). In subjects receiving the higher dose of atorvastatin, the reduction was more pronounced, 26.4%, from 36.5% ± 6.33% to 26.8% ± 4.96% ($P < .0004$).

DISCUSSION

HMG-CoA reductase inhibitors or statins constitute a class of compounds that are very effective in reducing plasma concentrations of CHOL and LDL-CHOL.[1-3] Two mechanisms of action have been suggested for this response. The expression of LDL receptors is induced by depletion of the intracellular CHOL pool following the inhibition of HMG-CoA reductase.[29,30] The clearance of circulating LDL is subsequently accelerated, leading to a reduction in the plasma levels of CHOL.[31,32] The direct clearance of TG-rich VLDL without conversion to LDL may also be increased via these receptors.[33-35] Alternatively, the reduction in intracellular CHOL may reduce the formation and secretion of VLDL into plasma.[33,34]

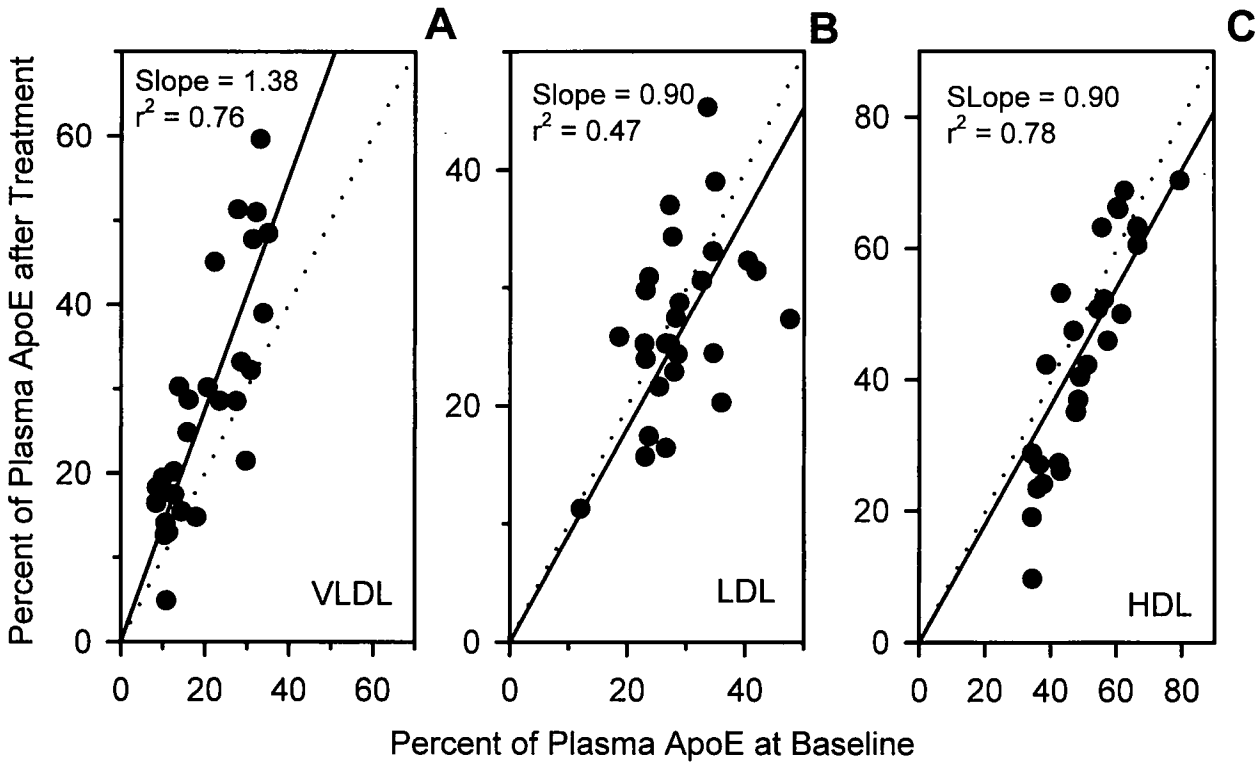

Fig 6. Effect of atorvastatin treatment on the distribution of apoE among the 3 major lipoprotein classes. (····) Line of identity (slope of 1), (—) regression line passing through the origin. (A) The percent of apoE in VLDL was consistently higher after treatment, with the mean value increased from 20.3% to 28.4% ($P < .0001$; Table 3). (B) The percent of apoE in LDL was slightly reduced after atorvastatin (mean, 28.9% to 27.0%, NS). (C) The percent of apoE in HDL was reduced in all individuals, with a mean value from 50.8% to 44.6% ($P < .0001$; Table 3).

Most studies on the efficacy of statins have been primarily limited to hypercholesterolemic patients with TG less than 350 mg/dL.[1-3] In addition to significant reductions in CHOL and LDL-CHOL, modest reductions in TG (10% to 20%) have also been reported.[4,5] Atorvastatin is a recently introduced synthetic HMG-CoA reductase inhibitor that has been reported to be very effective in reducing both TG and CHOL in patients with primary hypertriglyceridemia (TG range, 350 to 2,000 mg/dL). Mean reductions in TG of 26% to 46% have been reported after 4 weeks on either 5, 20, or 80 mg atorvastatin per day.[11]

In view of the concomitant reductions in CHOL and TG achieved with atorvastatin, we were interested in examining the effect of atorvastatin therapy on other apolipoproteins that are commonly associated with hypertriglyceridemia. Prior studies on the effect of HMG-CoA reductase inhibitors have been limited to changes in apoB, apoA-I, and Lp(a). Only a few studies have reported on changes in apoE, apoC-II, and apoC-III with reductase inhibitors. After 12 months of treatment with 10 to 20 mg pravastatin per day, Nishiwaki et al[19] reported no changes in apoC-III, apoE, and apoA-II. However, significant reductions (11%) in apoC-II from a mean value of 5.5 mg/dL to 4.9 mg/dL were found. In subjects receiving 40 mg/d lovastatin for 2 years, Alaupovic et al[20] reported an 18% reduction in plasma apoC-III and a 17% reduction in apoE. Data on plasma apoC-II were not available in their report. With fluvastatin, reductions in apoE levels (20%) have also been reported in patients with hypercholesterolemia.[21] ApoC-II and apoC-III were not examined in the study. Patients with elevated TG (TG > 350) were excluded from all 3 studies.

In the present study examining, for the first time, the effect of an HMG-CoA reductase inhibitor in patients with primary hypertriglyceridemia, highly significant reductions in plasma apoE were found, 37.6% with the lower dose of atorvastatin (20 mg/d) and 40.7% with the higher dose (80 mg/d). Reductions in plasma apoC-III of 16.2% and 23.4% with the low and high doses, respectively, were also statistically significant. Dose-dependent reductions in plasma apoC-II were also obtained in these hypertriglyceridemic patients (28.0% and 38.5% with the low and high doses, respectively). These reductions in apoCs and apoE were comparable to changes observed with fibric acid derivatives.[36,37] Significant reductions in apoB (30% to 35%) were achieved as previously reported,[11] while the minimal increases in apoA-I and apoA-II (1% to 5%) were not statistically significant.

We have recently reported on the use of FPLC to fractionate whole plasma into 20 fractions spanning the particle size range from VLDL to HDL using either fresh or frozen plasma.[22] In view of the fact that all of the samples have been kept frozen for at least 12 months, it was necessary to demonstrate that the lipid and apolipoprotein distribution have not been altered by storage. The mean recoveries for lipids and apolipoproteins from the eluted FPLC fractions were 73% to 101%. This was comparable to the recoveries reported by other groups using column chromatography to fractionate freshly isolated whole

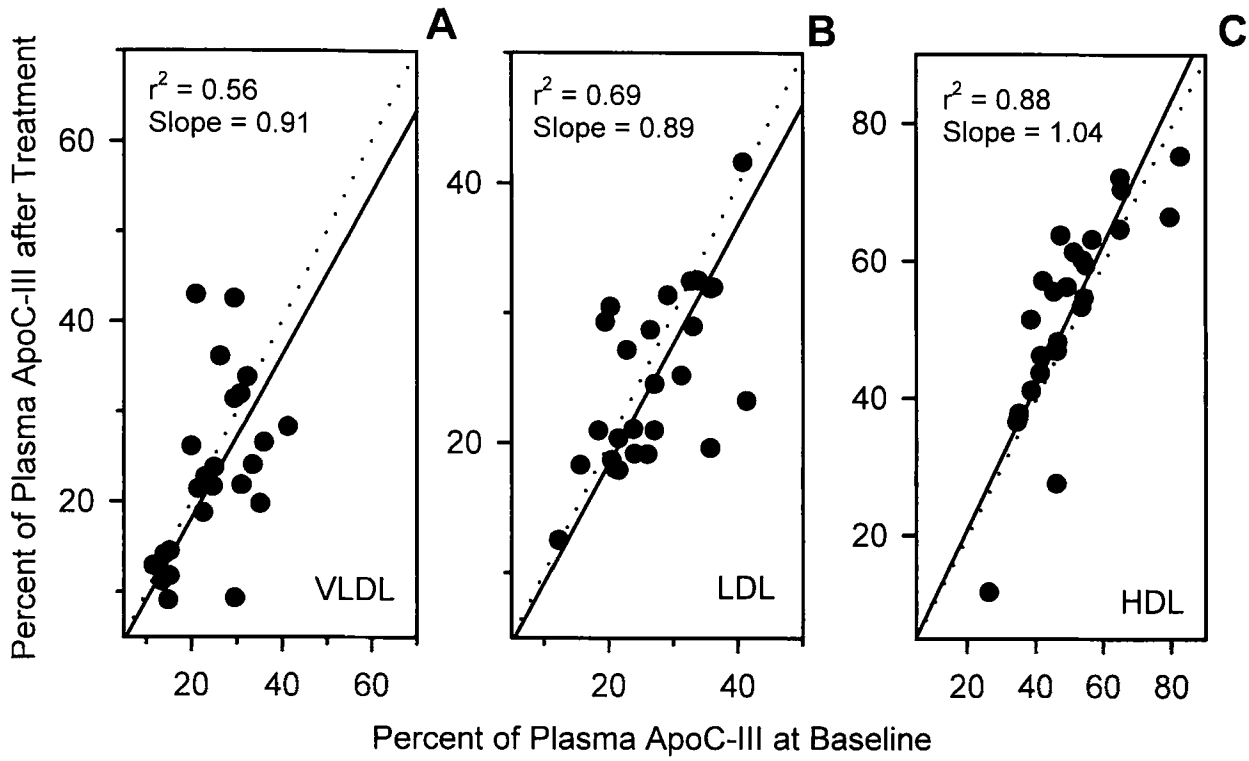

Fig 7. Effect of atorvastatin treatment on the distribution of apoC-III among the 3 major lipoprotein classes. (···) Line of identity (slope of 1), (—) regression line passing through the origin. The slope of the regression line has a range from 0.89 to 1.04, suggestive of minimal changes in the percent of apoC-III in any fraction including VLDL (A), LDL (B), or HDL (C) with atorvastatin therapy.

plasma.[23,38] The recoveries of lipids and apolipoproteins were not different between baseline samples and posttreatment samples (Table 3). The actual TG and CHOL concentrations in VLDL isolated by FPLC were comparable to values obtained by an ultracentrifuge at the Core Laboratory for Clinical Studies (Washington University, St Louis, MO) using freshly collected plasma. Comparability between the FPLC and ultracentrifuge methods for LDL-TG and LDL-CHOL has also been demonstrated.[22] Similar results have also been reported for HDL-TG and HDL-CHOL using FPLC and the precipitation method with dextran sulfate/$MgCl_2$[22] to recover the HDL fraction as standardized by the National Heart, Lung, and Blood Institute/Centers for Disease Control Lipid Standardization Program. Differences in the values reported in the present report and previously published values for the participants in this study reflect, in part, the differences in the instruments and reagents used for enzymatic determination of CHOL and TG.

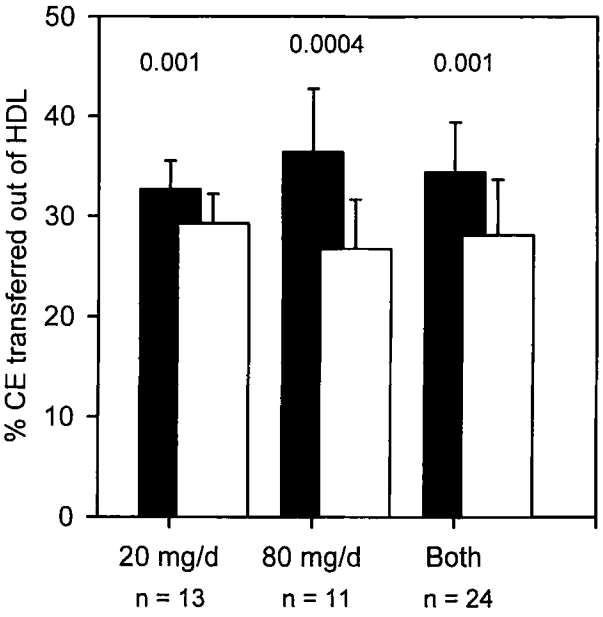

Fig 8. Effect of atorvastatin treatment on CETP activity. (■) Percent of CE transferred from HDL to LDL using baseline plasma as the source of CETP activity, (□) percent transferred with plasma obtained after atorvastatin treatment.

Several observations can be made from this analysis of the distribution of lipids and apolipoproteins among the 3 major lipoprotein classes. While there was a dose-dependent reduction in the plasma concentration of several parameters, the effect of atorvastatin on the distribution among plasma lipoproteins was the same for both the 20-mg/d and 80-mg/d doses. Despite the 32% to 39% reduction in total CHOL, the percent of plasma CHOL associated with the LDL fraction did not change. On the other hand, while we could not demonstrate a statistically significant increase in the actual concentration of HDL-CHOL, FPLC analysis indicated a redistribution of CHOL toward HDL with a significant increase in the percent of plasma CHOL associated with HDL (Fig 3). With the reduction in plasma TG achieved with atorvastatin, there was a greater fraction of plasma TG associated with HDL. The actual concentrations of

TG in all lipoprotein fractions including HDL were reduced with atorvastatin (Table 4).

The isotopic assay for maximal CETP activity used in the present report has been suggested to accurately reflect changes in CETP mass.[39,40] The reduction in CETP activity observed with atorvastatin is similar to the changes reported with other TG-lowering agents.[36] This may explain the higher percent of plasma CHOL being retained by HDL after therapy despite significant reductions in CHOL concentrations in all 3 lipoprotein classes. It has been suggested that a reduction in endogenous CHOL synthesis may downregulate CETP gene expression, leading to a reduction in CETP activity.[36] The reduced CETP activity with atorvastatin needs to be confirmed in freshly collected plasma that has not been frozen, as well as the determination of either CETP protein mass or CETP mRNA.

With respect to the redistribution of apoB, our data would indicate that atorvastatin therapy resulted in a reduction in the percent of apoB in VLDL and an increase in the percent of apoB associated with LDL. Thus, despite statistically significant reductions in apoB (number of apoB-containing particles), there are relatively more LDL particles than VLDL particles with atorvastatin therapy in this group of hypertriglyceridemic patients. This finding is consistent with other interventions that are effective in reducing plasma TG, such as weight loss.[18,41] Detailed analysis of the subfractions of apoB-containing lipoproteins defined by FPLC suggests the presence of larger particles with atorvastatin with respect to both the CHOL:apoB and TG:apoB ratio. This is consistent with a trend for larger LDL particle diameter as assessed by nondenaturing gradient gel electrophoresis. However, we could not demonstrate a statistically significant change in particle size in the present study. This may be due, in part, to the small number of subjects available and the large variability in LDL size inherent to this study population with a wide spectrum of hypertriglyceridemia, from 300 to 2,000 mg/dL at baseline. It is also possible that with atorvastatin therapy, more VLDL particles are converted to a subpopulation of LDL that is preferentially removed.

Our analyses also indicated that atorvastatin treatment resulted in a preferential loss of apoE from HDL, as illustrated in Fig 3D for 1 subject on the high dose, while the apoE content of VLDL was minimally affected. This increase in the percent of plasma apoE associated with VLDL can be demonstrated in all subjects treated with either the low or high dose (Fig 6). This preferential retention of apoE by VLDL during atorvastatin therapy could not be demonstrated with either apoC-II (Table 3) or apoC-III (Fig 7). Based on our controlled experiment, storage of whole plasma for 4 weeks at −80°C would be expected to have the same effect on apoE and apoC-III content in VLDL (Fig 3). While the absence of composition data in freshly collected plasma does not allow us to conclude unequivocally that atorvastatin therapy has a preferential effect on apoE distribution, this difference in the redistribution of apoE and apoCs cannot be ignored. Changes in the distribution of these apolipoproteins could not be demonstrated in frozen plasma from hypercholesterolemic patients treated with other reductase inhibitors (N.-A. Le et al, unpublished observation, 1996).

When the actual changes in apolipoprotein concentrations are taken into consideration, the reductions in apoB and apoE in VLDL result in an actual increase in the mean number of apoE molecules per VLDL particle, 0.032 versus 0.044. In contrast, the number of apoE molecules per LDL particle and per HDL particle (using apoA-I as the reference) was reduced with atorvastatin treatment, 0.023 versus 0.018 and 0.029 versus 0.015, respectively. This preferential association of apoE with TG-rich lipoproteins observed in this group of hypertriglyceridemic subjects after atorvastatin therapy may also explain the accelerated clearance of TG-rich remnants during postprandial lipemia in hypercholesterolemic patients treated with other statins.[21,35] Alternately, the preferential loss of apoE from the HDL fraction may reflect an accelerated catabolism of a subpopulation of apoE-rich HDL particles. These possibilities should be investigated further to better understand the mechanism of action of atorvastatin in vivo.

ACKNOWLEDGMENT

The authors are indebted to Debby Martinson for outstanding technical support in this project. Appreciation is also extended to all principal investigators and their staff who participated in this clinical trial for the recruitment and follow-up of the patients. The assistance of the staff of the Lipid Research Laboratory at St Louis University, in particular Shelley Meese, in the distribution of the samples is also greatly appreciated.

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