Effect of atorvastatin on plasma apoE metabolism in patients with combined hyperlipidemia

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Abstract **Atorvastatin, a synthetic HMG-CoA reductase inhibitor used for the treatment of hyperlipidemia and the prevention of coronary artery disease, significantly lowers plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) levels. It also reduces total plasma triglyceride and apoE concentrations. In view of the direct involvement of apoE in the pathogenesis of atherosclerosis, we have investigated the effect of atorvastatin treatment (40 mg/day) on in vivo rates of plasma apoE production and catabolism in six patients with combined hyperlipidemia using a primed constant infusion of deuterated leucine. Atorvastatin treatment resulted in a significant decrease (i.e., 30–37%) in levels of total triglyceride, cholesterol, LDL-C, and apoB in all six patients. Total plasma apoE concentration was reduced from 7.4 ± 0.9 to 4.3 ± 0.2 mg/dl (−38 ± 8%, $P < 0.05$), predominantly due to a decrease in VLDL apoE (3.4 ± 0.8 vs. 1.7 ± 0.2 mg/dl; −42 ± 11%) and IDL/LDL apoE (1.9 ± 0.3 vs. 0.8 ± 0.1 mg/dl; −57 ± 6%). Total plasma lipoprotein apoE transport (i.e., production) was significantly reduced from 4.67 ± 0.39 to 3.04 ± 0.51 mg/kg/day (−34 ± 10%, $P < 0.05$) and VLDL apoE transport was reduced from 3.82 ± 0.67 to 2.26 ± 0.42 mg/kg/day (−36 ± 10%, $P = 0.057$). Plasma and VLDL apoE residence times and HDL apoE kinetic parameters were not significantly affected by drug treatment. Percentage decreases in VLDL apoE concentration and VLDL apoE production were significantly correlated with drug-induced reductions in VLDL triglyceride concentration ($r = 0.99$, $P < 0.001$; $r = 0.88$, $P < 0.05$, respectively, $n = 6$).—Our results demonstrate that atorvastatin causes a pronounced decrease in total plasma and VLDL apoE concentrations and a significant decrease in plasma and VLDL apoE rates of production in patients with combined hyperlipidemia.**—Cohn, J. S., M. Tremblay, R. Batal, H. Jacques, L. Veilleux, C. Rodriguez, P. H. R. Barrett, D. Dubreuil, M. Roy, L. Bernier, O. Mamer, and J. Davignon. **Effect of atorvastatin on plasma apoE metabolism in patients with combined hyperlipidemia.** ***J. Lipid Res.*** **2002.** 43: **1464–1471.**

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Apolipoprotein E (apoE) is a 34 kDa glycoprotein that is synthesized and secreted by most human tissues, including the liver, large intestine, brain, kidney, spleen, adrenal gland, and lung (1, 2). It is associated in plasma with several classes of lipoproteins, including chylomicrons, VLDL, IDL, and a subclass of HDL (3). A pivotal role of apoE in plasma is to mediate hepatic and extrahepatic uptake of plasma lipoproteins by binding with high affinity to all members of the LDL-receptor family, including the LDL-receptor, the LDL-receptor-related protein (LRP), the VLDL receptor, GP330/megalin, and ApoER-2 (4). ApoE thus plays a central role in determining plasma cholesterol and triglyceride levels, a function that is best exemplified by the pronounced accumulation of cholesterol-rich lipoproteins and triglyceride-rich lipoprotein remnants in the plasma of both humans and mice lacking functional apoE (3, 5, 6). ApoE also modulates cellular cholesterol metabolism by facilitating cholesterol efflux (7). Endogenous apoE facilitates the transport of cholesterol from intracellular compartments to the cell membrane, or alternatively, secreted apoE can act as an extracellular acceptor of cellular cholesterol (8). ApoE-mediated cholesterol efflux from macrophages may be important in protecting these cells from the deleterious effects of cholesterol overload and transformation into atherosclerotic foam cells.

As a consequence of the aforementioned functions, apoE plays a critical role in the onset and development of atherosclerosis (9–11). This is a powerful protective role best exemplified by the severe hypercholesterolemia and pronounced atherosclerosis that occurs in apoE knockout ($apoE^{-/-}$) mice (6). Elevated levels of proatherogenic plasma lipoproteins clearly contribute to the development of arterial disease in these animals. This does not, how-

Abbreviations: apo, apolipoprotein; FPLC, fast protein-liquid chromatography; FTR, fractional transport rate; GC-MS, gas chromatography-mass spectrometry; IEF, isoelectric focusing; RT, residence time; TR, transport rate.

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ever, entirely explain the protective role of apoE. Secretion of apoE by macrophages is also important, since selective expression of apoE in macrophages of *apoE*$^{-/-}$ mice (through bone marrow transplantation or transgenic expression) significantly reduces the extent of atherosclerosis (12, 13). Even low levels of apoE made in insufficient quantities to correct the hyperlipidemia of *apoE*$^{-/-}$ mice (and not made in the liver or in macrophages) can protect against arterial atherosclerosis (14). ApoE may therefore have additional anti-atherogenic qualities, including inhibition of platelet aggregation (15), stimulation of NO production (16), reduction of smooth muscle cell migration and proliferation (17), and inhibition of lymphocyte proliferation (18). These latter functions are thought to be dependent on the ability of apoE to act as a cell signaling molecule, and to be independent of its role as a lipid transport protein (19).

Treatment of hyperlipidemic patients with 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors (or statins) has clearly been shown to reduce primary and secondary risk of cardiovascular morbidity and mortality (20, 21). Atorvastatin, a widely used synthetic HMG-CoA reductase inhibitor, significantly lowers plasma concentration of total cholesterol, LDL cholesterol (LDL-C), and apoB, and can also reduce plasma triglyceride levels (22, 23). Atorvastatin has also been shown to reduce plasma concentrations of apoE in patients with hypercholesterolemia (24) or hypertriglyceridemia (25). This may be due to reduced production or increased catabolism of plasma apoE. Although a number of previous studies have investigated the plasma kinetics of apoE in normolipidemic and hyperlipidemic subjects (26–32), no previous work has investigated the effect of drug treatment on plasma or lipoprotein apoE kinetics. We have therefore carried out the present study using a primed constant infusion of deuterated leucine, in which plasma apoE metabolism, i.e., rates of plasma apoE transport (production) and fractional catabolism (i.e., residence time) were determined in combined hyperlipidemic patients treated with atorvastatin.

METHODS

Patients and treatment

Six male patients with combined hyperlipidemia were selected from our lipid clinic. Each patient gave their informed consent to participate in the study, which was approved by the Ethics Committee of the Clinical Research Institute of Montreal. At screening, they had a fasting plasma triglyceride concentration greater than 2.3 mmol/l (200 mg/dl), but less than 9.10 mmol/l (800 mg/dl), and an LDL-C concentration greater than 4.1 mmol/l (160 mg/dl). At screening, their plasma triglyceride concentration was 3.48 ± 0.23 mmol/l and their LDL-C was 5.21 ± 0.23 mmol/l. Their age (mean ± SE) was 47 ± 6 years and BMI was 26.8 ± 0.9. Two patients had an apoE-4/3 phenotype (numbers 2 and 3 in Tables) and four had an apoE-3/3 phenotype. They had no evidence nor history of diabetes mellitus, or liver or thyroid disease, and at screening were not taking medications known to affect plasma lipid metabolism. Patients were studied on two occasions: once while being treated with atorvastatin (40 mg/day) and then on a second occasion while not taking medication. For five patients, the atorvastatin treatment period (a minimum of six months) preceeded the non-treatment period (a minimum of 5 weeks). For one patient (number 3 in Tables), the first infusion was carried out while untreated, and the second infusion was carried out after 6 weeks of atorvastatin therapy.

Protocol for apoE kinetic study

After a 12-h overnight fast, patients were given a primed constant intravenous infusion of deuterium-labeled leucine (L-[D_3]leucine 98%, Cambridge Isotope Laboratories, MA), as described previously (33, 34). They were injected via a needle inserted into a left forearm vein with 10 μmol per kg body weight of L-[D_3]leucine dissolved in physiological saline, followed by a 12-h constant infusion (given by peristaltic pump) of 10 μmol L-[D_3]leucine per kg per h. Subjects remained fasted during the infusion but had free access to drinking water. Blood samples (20 ml) were collected from an antecubital vein of the right arm at regular intervals (0, 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h) in tubes containing EDTA to a final concentration of 0.1%. Plasma was immediately separated by centrifugation at 3,500 rpm for 15 min at 4°C. An antimicrobial agent (sodium azide) and a protease inhibitor (aprotinin) were added to plasma samples to give a final concentration of 0.02% and 1.67 μg/ml, respectively.

Isolation of lipoproteins and apolipoproteins

VLDL, IDL together with LDL, and HDL, were isolated from 5 ml plasma by sequential ultracentrifugation in an XL-90 ultracentrifuge using a 50.4 Ti rotor (Beckman) at 50,000 rpm for 10 h, at densities (d) of 1.006, 1.063, and 1.21 g/ml, respectively. Total lipoproteins were isolated from plasma by ultracentrifugation (50,000 rpm, 10 h) of 2 ml of plasma, adjusted to d = 1.25 g/ml with KBr. Lipoproteins were recovered in the supernate by tube-slicing. ApoE was isolated from VLDL, HDL, and total plasma lipoproteins (d < 1.25 g/ml fractions) by preparative isoelectric focusing (IEF) on 7.5% polyacrylamide-urea (8M) gels (pH gradient 4–7), as described previously (35). Before IEF separation of HDL and the d < 1.25 g/ml fractions, they were dialyzed against 10 mM ammonium bicarbonate, preincubated with cysteamine (β-mercaptoethylamine, Sigma-Aldrich) in a ratio of 6 mg for every milligram of protein for 4 h at 37°C, and then delipidated. The aim of cysteamine treatment was to separate apoE from isoforms of apoA-I, which normally co-migrate to the same position on IEF gels. Cysteamine treatment causes an amino group to bind to the single cysteine residue of apoE3. ApoA-I and apoE-4 are not affected since they do not contain cysteine. Cysteamine-modified apoE-3 consequently migrates to a higher position in IEF gels due to its increased positive charge (35). VLDL samples were delipidated, but were not treated with cysteamine prior to electrophoresis. Coumassie blue staining was used to identify the position of apolipoproteins in gels after electrophoresis. The VLDL apoE-3 band was analyzed for enrichment in all six patients. In apoE-3/3 patients, approximately 80% of VLDL apoE migrated in the E3 position and was analyzed. In apoE4/3 patients, approximately 40% of VLDL apoE migrated in the E3 position and was analyzed. This meant that samples from apoE-4/3 and apoE-3/3 patients were analyzed slightly differently. Importantly, however, the type and amount of VLDL apoE analyzed for a given patient was the same, whether he was on or off statin therapy.

Plasma lipids and apolipoproteins

ApoE phenotypes were determined by isoelectric focusing of delipidated VLDL (35). Plasma and lipoprotein fractions were assayed for total (free and esterified) cholesterol and triglyceride

with a COBAS MIRA-S automated analyzer (Hoffman-LaRoche) using enzymatic reagents. HDL-C was determined by assaying cholesterol in the supernatant after precipitation of apoB-containing lipoproteins in the d > 1.006 g/ml fraction with heparin/manganese. LDL-C was calculated as d > 1.006 g/ml cholesterol minus cholesterol in HDL. Plasma apoA-I and apoB were measured by nephelometry on a Behring Nephelometer 100 (Behring) using Behring protocol and reagents. Plasma and lipoprotein apoE concentrations were measured with an ELISA developed in our laboratory (36) using immunopurified polyclonal antibody (Biodesign, Kennebunk, ME). Recovery of apoE after sequential ultracentrifugation was determined as a percentage: (VLDL apoE + IDL/LDL apoE + HDL apoE + d > 1.21 g/ml apoE) × 100/total plasma apoE. ApoE recovery was 74.5 ± 2.0% for plasma isolated from untreated patients and 71.3 ± 1.5% for plasma isolated from treated patients. Final lipoprotein apoE concentrations were corrected proportionately to give 100% recovery.

Determination of isotopic enrichment

Apolipoprotein bands, as well as blank (non-protein containing) gel slices, were excised from polyacrylamide gels as described previously (35). The band corresponding to non-sialylated apoE3 was analyzed in VLDL. For HDL and d < 1.25 g/ml fractions, apoE isoforms migrated together as one band due to cystamine treatment. Each slice was added to a borosilicate sample vial containing 600 μl of 6 N HCL, and an internal standard (250 ng norleucine, Sigma-Aldrich) dissolved in 50 μl double distilled water. Gel slices were hydrolyzed at 110°C for 24 h, cooled to −20°C for 20 min, and centrifuged at 3,500 rpm for 5 min. Free amino acids in the hydrolysate were separated from precipitated polyacrylamide, purified by cation exchange chromatography using AG 50 W-X8 resin (BioRad), and derivatized by treatment with 200 μl of acetyl chloride-acidified 1-propanol (1:5, v/v) for 1 h at 100°C, and 50 μl of heptaflurobutyric anhydride (Supelco) for 20 min at 60°C (33). Plasma amino acids were also separated by cation exchange chromatography and derivatized to allow for the determination of plasma leucine isotopic enrichment. Enrichment of samples with deuterium-labeled leucine was measured by gas chromatography/mass spectrometry (Hewlett-Packard, 5988 GC-MS) using negative chemical ionization and methane as the moderator gas. Selective ion monitoring at m/z =352 and 349 (ionic species corresponding to derivatized deuterium-labeled and derivatized non-deuterium-labeled leucine, respectively) was performed, and tracer to tracee ratios were derived from isotopic ratios for each sample. Tracer to tracee ratios were corrected for background leucine in gel slices (i.e., trace amounts of leucine introduced during the amino acid purification and derivitization procedures) by estimating the amount of leucine in processed blank gel slices relative to the norleucine internal standard. Background leucine varied from 0.5% to 10% of total leucine recovered from each gel slice containing apoE.

Kinetic analysis

Stable isotope enrichment curves for apoE in total plasma lipoproteins (d < 1.25 g/ml), VLDL, and HDL were fitted to a three compartment model using SAAM II computer software (SAAM II institute, WA). The first compartment represented the plasma amino acid precursor pool. The second compartment was a delay compartment, which accounted for the synthesis, assembly, and secretion of apolipoprotein. The third compartment was the plasma protein compartment. Plasma leucine enrichment (measured eight times during the course of the infusion experiment) was used as a measure of precursor pool enrichment. Mean (± SE) plasma leucine tracer to tracee ratio was 11.4 ± 0.5% in untreated patients and was 10.9 ± 0.5% in treated patients. Modeling of tracer to tracee ratio data allowed for the determination of fractional transport rates (FTR) (i.e., the fraction of protein pools being renewed per day). Residence time (RT) was calculated as the reciprocal of FTR (1/FTR), and transport rate (TR) was calculated (in mg/kg.day) as:

$$TR = FTR\ (\text{pools/day}) \times \text{apolipoprotein pool size (mg)} \div \text{body weight (kg)}$$

where: pool size = plasma concentration (mg/dl) × plasma volume (0.045 l/kg).

For VLDL and HDL, apoE transport rates were a reflection of the amount of apoE becoming associated with either plasma VLDL or HDL per unit time. The majority of this apoE in the fasted state was newly synthesized and was acquired directly from tissue. This parameter, however, also measured apoE that was transported into VLDL or HDL indirectly from other lipoproteins due to lipoprotein conversion or apolipoprotein exchange. In the case of total plasma lipoprotein apoE, transport rates were a reflection of the amount of apoE entering the circulation per unit time and were thus a measure of overall tissue synthesis and secretion (i.e., production) of plasma apoE.

Statistical analysis

Statistical significance of differences between mean values was assessed by paired t-tests using SigmaStat software (Jandel Scientific, CA). Pearson correlation coefficients (r) were calculated to describe the correlation between different kinetic and mass parameters.

RESULTS

Atorvastatin treatment was associated with a decrease in the plasma concentration of total triglyceride, cholesterol, VLDL-C, LDL-C, and apoB in all six patients. These parameters were on average reduced by ∼35%, except for VLDL-C, which was reduced by 60% (**Table 1**). VLDL triglyceride concentration was 2.20 ± 0.39 mg/dl in untreated patients and 1.36 ± 0.16 mg/dl in treated patients (−33 ± 9%, P = 0.064). VLDL apoB concentration was 15.5 ± 2.3 mg/dl in untreated patients and 9.3 ± 1.6 mg/dl in treated patients (−38 ± 11%, P < 0.05). There was no significant difference in HDL-C or plasma apoA-I levels. Total plasma apoE concentrations were also significantly decreased (−38 ± 8%, P < 0.05). This reduction was predominantly due to less apoE in the VLDL and IDL/LDL fractions (**Table 2**). The one patient (number 3) who was studied in the order "untreated–treated," and who was therefore on atorvastatin treatment for a shorter period of time (6 weeks vs. 6 months), had a similar decrease in plasma lipids and apolipoproteins as the other patients. The two patients (numbers 2 and 3) with an apoE-4/3 phenotype responded to therapy, even though in men the apoE-4 allele is generally associated with a poorer response to statin therapy and the apoE-2 allele is associated with a better response (37).

Deuterated leucine enrichment of apoE in VLDL, HDL, and in total plasma lipoproteins of patients treated and untreated with atorvastatin is shown in **Fig. 1**. Mean (± SE) data are shown for the six patients combined. For each patient, tracer to tracee ratios were expressed as a

TABLE 1. Plasma lipid and apolipoprotein concentrations in patients untreated or treated with atorvastatin

	Total Triglyceride	Total Cholesterol	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol	ApoB	ApoA-I
			mmol/l				*mg/dl*
Untreated							
1	2.36	8.34	0.73	6.28	1.33	207	107
2	2.16	6.86	0.93	4.73	1.20	151	134
3	3.19	6.81	1.28	4.33	1.20	201	116
4	1.42	8.02	0.48	6.77	0.78	229	119
5	2.06	7.53	0.76	6.06	0.71	192	89
6	4.17	7.18	1.98	3.91	1.28	177	112
Mean ± SE	2.56 ± 0.40	7.46 ± 0.26	1.03 ± 0.22	5.35 ± 0.48	1.08 ± 0.11	193 ± 11	113 ± 6
Treated							
1	1.61	5.49	0.32	4.00	1.17	119	111
2	2.09	4.80	0.67	2.86	1.27	133	113
3	1.79	3.89	0.44	2.10	1.35	103	119
4	0.94	5.16	0.07	4.10	0.99	127	130
5	1.91	5.26	0.43	4.12	0.71	149	90
6	1.64	4.46	0.39	3.11	0.96	109	108
Mean ± SE	1.66 ± 0.16	4.84 ± 0.24	0.39 ± 0.08	3.38 ± 0.34	1.08 ± 0.10	123 ± 7	112 ± 5
Percent change[a]	−30 ± 9%	−35 ± 2%	−60 ± 9%	−37 ± 4%	1 ± 7%	−35 ± 6%	0 ± 3%
Significance[b]	0.065	< 0.001	< 0.05	< 0.001	ns	< 0.01	ns

Values are for individual patients (numbered 1–6). Each value is the mean of five measurements (at 3 h intervals) during the infusion experiment.
[a] Percent change due to treatment was calculated for each patient and then averaged.
[b] Statistical significance between untreated and treated values was determined by paired Student's *t*-test and is given as a *P* value; ns = not significant.

percentage relative to that of plasma leucine in order to normalize the results. Data could not be obtained for apoE in the IDL/LDL fraction. This was due to the fact that only faint bands of apoE could be observed after electrophoresis of IDL/LDL. Even though the plasma concentration of apoE in the IDL/LDL fraction was the same or greater than that of the HDL fraction (Table 2), the amount of apoE recovered from IDL/LDL was considerably less. We believe that this was due to the relatively large amount of apoB present, which interfered with the recovery of apoE during the delipidation stage of sample preparation. As we have shown previously, the rate of enrichment of VLDL apoE and total plasma lipoprotein apoE was curvilinear, whereas that of HDL apoE was linear (31). In general, apoE tracer to tracee ratios were higher in VLDL and HDL during drug treatment, reflecting increased (though not significantly higher) fractional turnover of these proteins during treatment. Kinetic parameters were determined by computer modeling (see Methods) and mean (± SE) data are presented in **Table 3**. For the determination of HDL apoE kinetic parameters, in vivo HDL apoE concentrations were taken to be the sum of apoE in HDL and in the d > 1.21 g/ml fraction, as described previously (31). This is based on the observation that: *1*) FPLC separation of plasma lipoproteins, which avoids the high sheer stress of UTC and therefore

TABLE 2. Total plasma and lipoprotein apoE concentrations in patients untreated or treated with atorvastatin

	VLDL ApoE	IDL/LDL ApoE	HDL ApoE	d > 1.21 g/ml ApoE	Total Plasma ApoE
Untreated					
1	2.8	2.5	1.4	1.0	7.8
2	2.4	1.4	0.4	1.0	5.2
3	3.8	1.1	0.5	1.3	6.7
4	1.9	3.0	1.0	1.4	7.3
5	2.2	1.5	1.0	1.4	6.1
6	7.1	1.9	0.7	1.8	11.5
Mean ± SE	3.4 ± 0.8	1.9 ± 0.3	0.8 ± 0.2	1.3 ± 0.1	7.4 ± 0.9
Treated					
1	1.6	0.7	0.9	1.1	4.2
2	2.2	0.9	0.6	0.9	4.6
3	1.7	0.3	0.5	0.8	3.3
4	1.0	1.1	1.1	1.1	4.2
5	1.9	0.9	0.8	1.3	4.9
6	1.6	0.7	0.9	1.4	4.6
Mean ± SE	1.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	4.3 ± 0.2
Percent change[a]	−42 ± 11%	−57 ± 6%	5 ± 12%	−17 ± 6%	−38 ± 8%
Significance[b]	0.086	<0.01	ns	<0.05	<0.05

Values (in mg/dl) are for individual patients (numbered 1–6). Each value is the mean of five measurements made at 3 h intervals during the infusion experiment. Lipoprotein apoE concentrations were corrected to give 100% recovery.
[a] Percent change due to treatment was calculated for each patient and then averaged.
[b] Statistical significance between untreated and treated values was determined by paired Student's *t*-test and is given as a *P* value; ns = not significant.

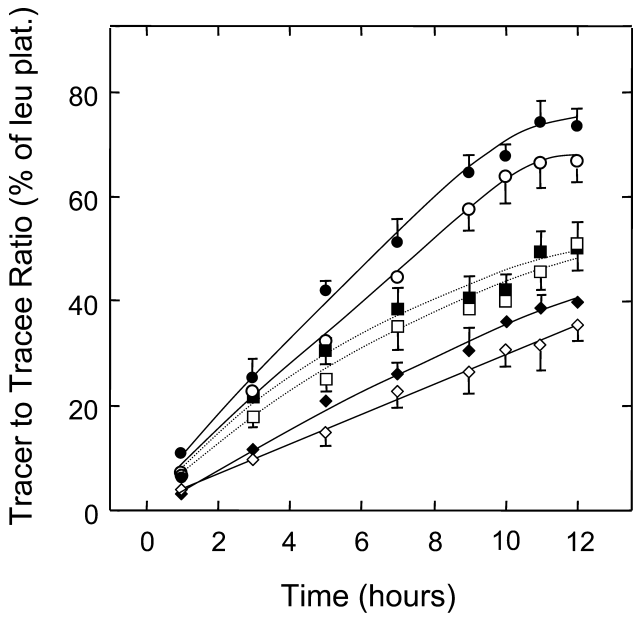

Fig. 1. Enrichment of VLDL-apolipoprotein E (apoE) (circles), HDL-apoE (diamonds) and total plasma apoE (squares) with deuterium-labeled leucine in atorvastatin untreated (open symbols) and treated patients (closed symbols). Error bars represent SEM. ApoE tracer to tracee ratios were expressed as a percentage relative to the enrichment of plasma leucine in order to combine results from individual patients.

does not result in dissociation apoE from lipoproteins, has demonstrated that patients with combined hyperlipidemia (n = 12) with a plasma apoE concentration of 8.8 ± 1.0 mg/dl have an FPLC-isolated HDL apoE concentration of 2.0 ± 0.3 mg/dl (36); combining HDL and d > 1.21 g/ml apoE for untreated patients in the present study (total plasma apoE = 7.4 mg/dl) gives a similar concentration (i.e., 2.2 ± 0.2 mg/dl). *2*) Separation of plasma apoE-containing HDL-sized particles by two-dimensional gel electrophoresis has demonstrated that apoE is associated with apoA-I-containing lipoproteins, and with lipoproteins without apoA-I, and is also associated with certain plasma proteins in a non-lipidated form, which are isolated at d > 1.21 g/ml (38, 39). ApoE in all these fractions can transfer to TRL in the postprandial period and appears to behave (at least kinetically) like HDL apoE (38). *3*) ApoE was isolated from the d > 1.21 g/ml fraction by affinity chromatography in a single untreated patient and its kinetics were identical to that of HDL apoE and not VLDL apoE (data not shown).

Reduced total plasma apoE concentration in atorvastatin-treated patients was associated with a significant decrease in the rate of transport of total plasma lipoprotein apoE (−34%, $P < 0.05$), with no significant change in plasma lipoprotein apoE residence time (Table 3). Mean plasma apoE concentrations and apoE transport rates after atorvastatin treatment (Table 3) were virtually identical to those found previously in normal, healthy subjects (i.e., from ref. 31, for healthy normolipidemics: apoE conc. = 4.28 ± 0.31 mg/dl, apoE TR = 2.94 ± 0.78 mg/kg/day, apoE RT = 0.85 ± 0.19 days).

VLDL apoE concentration was reduced (−42%) by drug treatment, and this was associated with a decrease in VLDL apoE transport (−36%, $P = 0.057$). Percentage decrease in VLDL apoE concentration was significantly correlated with percentage decrease in VLDL triglyceride concentration ($r = 0.99$, $P < 0.001$), and as shown in **Fig. 2**, decrease in VLDL apoE transport was also significantly correlated with percentage decrease in VLDL triglyceride concentration ($r = 0.88$, $P < 0.05$). Percentage reduction

TABLE 3. Plasma apoE kinetic parameters in patients untreated or treated with atorvastatin

	VLDL ApoE			HDL ApoE			Total Plasma Lipoprotein ApoE[a]		
	TR	RT	Conc.	TR	RT	Conc.	TR	RT	Conc.
	mg/kg.day	*days*	*mg/dl*	*mg/kg.day*	*days*	*mg/dl*	*mg/kg.day*	*days*	*mg/dl*
Untreated									
1	3.56	0.35	2.80	0.91	1.22	2.45	5.18	0.68	7.77
2	4.25	0.25	2.41	0.83	0.79	1.45	5.54	0.42	5.22
3	5.96	0.29	3.82	0.88	0.91	1.79	4.64	0.65	6.66
4	1.51	0.57	1.92	0.66	1.62	2.36	3.13	0.96	7.26
5	2.55	0.39	2.18	1.05	0.99	2.37	4.02	0.68	6.06
6	5.08	0.63	7.10	0.67	1.66	2.48	5.52	0.93	11.47
Mean ± SE	3.82 ± 0.67	0.41 ± 0.06	3.37 ± 0.79	0.83 ± 0.06	1.20 ± 0.15	2.15 ± 0.17	4.67 ± 0.39	0.72 ± 0.08	7.41 ± 0.89
Treated									
1	2.15	0.70	1.56	0.88	1.01	1.98	3.34	0.57	4.20
2	4.20	0.90	2.20	0.81	0.83	1.48	5.34	0.39	4.58
3	2.10	0.30	1.70	0.63	0.89	1.25	1.85	0.80	3.29
4	1.15	1.10	0.97	1.64	0.59	2.16	2.81	0.68	4.24
5	1.91	0.90	1.88	0.83	1.14	2.11	2.67	0.82	4.86
6	2.06	0.70	1.61	0.83	1.23	2.27	2.22	0.93	4.59
Mean ± SE	2.26 ± 0.42	0.35 ± 0.03	1.65 ± 0.17	0.94 ± 0.14	0.95 ± 0.09	1.88 ± 0.17	3.04 ± 0.51	0.70 ± 0.08	4.29 ± 0.22
Percent change[b]	−36 ± 10%	−8 ± 11%	−42 ± 11%	20 ± 27%	−15 ± 11%	−13 ± 4%	−34 ± 10%	−1 ± 8%	−38 ± 8%
Significance[c]	0.057	ns	0.086	ns	ns	0.022	0.025	ns	0.018

Values are for individual patients (numbered 1–6). TR, transport rate; RT, residence time.
[a] Total plasma lipoprotein apoE represents apoE isolated in the d < 1.25 g/ml fraction.
[b] Percent change due to treatment was calculated for each patient and then averaged.
[c] Statistical significance between untreated and treated values was determined by paired Student's *t*-test and is given as a *P* value; ns = not significant.

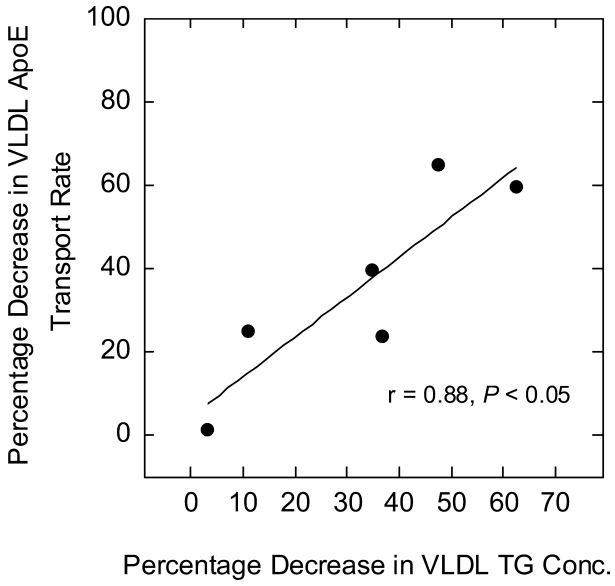

Fig. 2. Relationship between percentage reduction in VLDL triglyceride concentration due to drug treatment and percentage decrease in the rate of VLDL apoE transport.

in VLDL apoE residence time was not significantly related to decrease in VLDL triglyceride concentration ($r = 0.44$, $P = 0.39$). Percentage decreases in VLDL-C and VLDL apoB concentrations were also significantly correlated with percentage decrease in VLDL apoE concentration ($r = 0.88$, $P < 0.05$), but their positive relationship with VLDL apoE production did not reach statistical significance ($r = 0.61$, P = 0.20 and $r = 0.77$, P = 0.07, respectively). Drug-induced decreases in total lipoprotein and VLDL apoE transport did not correlate with changes in LDL or HDL-C levels. No consistent change due to drug treatment was observed in VLDL residence times (four patients had a decrease in VLDL apoE residence time and two had an increase). A small but significant decrease in HDL apoE concentration was associated with inconsistent changes in HDL apoE kinetic parameters; HDL apoE transport increased in four patients and decreased in two patients, while HDL apoE residence time decreased in four patients and increased in two patients.

DISCUSSION

The results of the present study have shown that atorvastatin treatment of patients with combined hyperlipidemia causes a pronounced decrease in total plasma and VLDL apoE concentrations and a significant decrease in total lipoprotein and VLDL apoE rates of transport. No evidence was found for a consistent effect of atorvastatin on total plasma or VLDL apoE residence time, suggesting that the overall effect of atorvastatin treatment on plasma apoE metabolism was to affect rates of apoE production rather than catabolism.

An in vivo reduction of plasma apoE production by atorvastatin is consistent with previous studies in experimental animals and cultured cells, demonstrating the ability of HMG-CoA reductase inhibitors to reduce apoE synthesis and secretion in different tissues. For example, Mitchell et al. have shown that simvastatin given orally to rats for 8 days resulted in a 37% decrease in plasma apoE levels and a 37% decrease in hepatic mRNA levels (40). In genetically hypercholesterolemic (RICO) rats, Felgines et al. found that simvastatin administration induced a significant decrease in hepatic apoE mRNA, despite no change in plasma apoE levels (41). Ribeiro et al. found that incubation of rat hepatocytes in primary culture with simvastatin (10^{-7} M for 24 h) caused a decrease in apoE synthesis and secretion, but no change in hepatocyte mRNA (42), and finally, Cignarella et al. showed that lovastatin treatment caused apoE mRNA levels to increase, but caused apoE secretion into the medium to decrease in both cholesterol-loaded and non-loaded human monocyte-derived macrophages (43). Taken together, these data demonstrate that HMG-CoA reductase inhibitors reduce tissue production of apoE, although it is unclear whether this occurs at a transcriptional, post-transcriptional, or post-translational level.

We have previously demonstrated that patients with increased levels of plasma triglyceride have increased levels of plasma and VLDL apoE production (31). Reduction in the plasma concentration of VLDL by atorvastatin, particularly VLDL triglyceride and apoE, is in turn associated with a pronounced decrease in VLDL apoE production (Table 3). Although data have been obtained from only 6 patients, it is evident that the extent of reduction in VLDL apoE concentration and in VLDL apoE production due to drug treatment were both strongly related to the extent of VLDL triglyceride lowering (Fig. 2). These results lend support to the concept that hepatic secretion of apoE is linked to hepatic VLDL triglyceride production, as suggested by studies in experimental animals. These experiments have consistently demonstrated that decreased hepatic apoE expression is associated with reduced VLDL production in the liver, and increased apoE expression leads to increased VLDL production (44–49). In humans, however, it is unclear to what extent apoE production in the liver controls VLDL production, or alternatively to what extent hepatic VLDL production is responsible for determining apoE production. It is likely that both scenarios occur, depending on the physiological circumstances and on the genetic background of the individual. One example where an interaction of gene and environment plays a critical role in determining plasma apoE metabolism and plasma apoE production is in patients with type III hyperlipoproteinemia. These patients have a severe apoE mutation or are homozygous for a functionally impaired apoE isoform (i.e., apoE-2). They have increased levels of plasma triglyceride and cholesterol, and are at increased risk of coronary disease. They are dyslipidemic not only because they have dysfunctional apoE, but because there is a second environmental factor that precipitates their hyperlipidemic condition. They subsequently have markedly increased levels of plasma and VLDL apoE, and as well as having impaired VLDL apoE catabolism,

they have an 7-fold increase in VLDL apoE production (31).

In addition to having a pronounced effect on plasma VLDL apoE concentrations, atorvastatin treatment resulted in a significant decrease in levels of IDL/LDL apoE (Table 2). Although concentration of IDL/LDL apoE in untreated patients was only half that of VLDL, the percentage reduction in IDL/LDL apoE due to drug therapy was as great, if not greater, than that observed for VLDL. These results are consistent with those of Dallongeville et al. (24), who showed a significant (25%) decrease in LpE:B levels in hypercholesterolemic patients given 10 mg/day of atorvastatin for 16 weeks, and are also consistent with the results of Le et al. (25), who observed a 19% decrease in VLDL apoE and a 44% decrease in IDL/LDL apoE (isolated by FPLC) in primary hypertriglyceridemic patients given 20 or 80 mg/day atorvastatin for 4 weeks. Decreased levels of IDL/LDL apoE could be a result of reduced hepatic VLDL apoE production, reduced conversion of VLDL apoE to IDL/LDL apoE, and/or increased IDL/LDL apoE clearance. Unfortunately, for technical reasons (due to the large amount of apoB relative to apoE in the IDL/LDL fraction), we were unable to isolate sufficient amounts of IDL/LDL apoE in the present study, and we were therefore unable to derive kinetic parameters for this fraction.

It is seemingly paradoxical that apoE is an anti-atherogenic protein (4–19) and yet hypertriglyceridemic patients at increased risk of coronary artery atherosclerosis have increased levels of plasma and VLDL apoE, as well as increased rates of plasma and VLDL apoE production (31). This apparent paradox is somewhat analogous to the raised levels of insulin found in patients with diabetes, or the elevated levels of leptin observed in patients with obesity. In all these situations, an important metabolic regulator is being overexpressed or is present in increased quantities, in order to overcome a stressed metabolic situation. In the present context, it is evident that by inhibiting HMG-CoA reductase and reducing endogenous levels of cholesterol synthesis, atorvastatin leads to a decrease in already elevated levels of plasma and VLDL apoE and helps to normalize rates of plasma and VLDL apoE production.

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