Effects of the cholesteryl ester transfer protein inhibitor torcetrapib on VLDL apolipoprotein E metabolism

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Abstract **Cholesteryl ester transfer protein (CETP) inhibition leads to changes in lipoprotein metabolism. We studied the effect of the CETP inhibitor torcetrapib on VLDL apolipoprotein E (apoE) metabolism. Subjects, pretreated with atorvastatin (n = 9) or untreated (n = 10), received placebo followed by torcetrapib (4 weeks each). After each treatment, subjects underwent a primed-constant infusion of D_3-leucine to determine the VLDL apoE production rate (PR) and fractional catabolic rate (FCR). Torcetrapib alone reduced the VLDL apoE pool size (PS) (−28%) by increasing the VLDL apoE FCR (77%) and leaving the VLDL apoE PR unchanged. In subjects pretreated with atorvastatin, torcetrapib increased the VLDL apoE FCR (25%) and PR (21%). This left the VLDL apoE PS unchanged but increased the VLDL apoE content, likely enhancing VLDL clearance and reducing LDL production in this group. Used alone, torcetrapib reduces the VLDL apoE PS by increasing the apoE FCR while leaving the VLDL apoE content unchanged. In contrast, torcetrapib added to atorvastatin treatment increases both the VLDL apoE FCR and PR, leaving the VLDL apoE PS unchanged. Adding torcetrapib to atorvastatin treatment increases the VLDL apoE content, likely leading to decreased conversion of VLDL to LDL, reduced LDL production, and lower levels of circulating VLDL and LDL.—**Millar, J. S., M. E. Brousseau, M. R. Diffenderfer, P. H. R. Barrett, F. K. Welty, J. S. Cohn, A. Wilson, M. L. Wolfe, C. Nartsupha, P. M. Schaefer, A. G. Digenio, J. P. Mancuso, G. G. Dolnikowski, E. J. Schaefer, and D. J. Rader. **Effects of the cholesteryl ester transfer protein inhibitor torcetrapib on VLDL apolipoprotein E metabolism. *J. Lipid Res.* 2008.** 49: **543–549.**

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Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl ester from HDL to VLDL. CETP inhibitors alter lipoprotein metabolism by reducing the CETP-mediated transfer of cholesteryl ester and triglyceride between HDL and VLDL (1). The net result of this inhibition is the accumulation of cholesteryl ester in HDL and triglyceride in VLDL, thus changing both the mass and composition of these lipoproteins (2, 3). The change in lipoprotein mass and composition might be expected to alter the distribution of exchangeable apolipoproteins, including apolipoprotein E (apoE), between VLDL and HDL, because the affinity of these proteins for lipoproteins is affected by lipoprotein size and composition (4). Redistribution of apoE among lipoproteins would be expected to influence VLDL metabolism, because apoE is a ligand for the clearance of apoB-containing lipoproteins from plasma.

In addition to influencing the clearance of triglyceride-rich lipoproteins (chylomicrons and VLDL) from plasma, apoE has been hypothesized to influence the conversion of VLDL to LDL (5). This has been proposed to occur as a result of the preferential removal of apoE-rich VLDL and intermediate density lipoprotein (IDL) precursors of LDL. Consistent with this hypothesis, we previously reported that the apoE content of newly secreted VLDL can influence the LDL production rate (PR), with a reduced VLDL apoE content being associated with increased LDL apoB-100 production (6).

The goal of the present study was to determine the effect of torcetrapib treatment on the metabolism of apoE. We conducted kinetic studies to define the mechanism(s)

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by which torcetrapib, alone or on a background with the HMG-CoA reductase inhibitor atorvastatin, influences lipoprotein metabolism (2, 3). The results show that CETP inhibition with torcetrapib alone significantly reduces VLDL apoE levels, primarily as a result of an enhanced clearance. Subjects treated with torcetrapib while on the background of atorvastatin treatment also showed enhanced apoE clearance, and this was counteracted by a significant increase in the PR of apoE within the VLDL fraction, resulting in no change in the VLDL apoE concentration. These changes in apoE metabolism in response to CETP inhibition with torcetrapib likely contributed to the reduction in the concentrations of apoB-containing lipoproteins observed in these subjects.

METHODS

Subjects

Subjects were recruited at the University of Pennsylvania (Philadelphia, PA) and at Tufts-New England Medical Center (Boston, MA). Subjects met the following eligibility criteria: age between 18 and 70 years, HDL-cholesterol (HDL-C) < 40 mg/dl, triglycerides < 400 mg/dl, LDL-cholesterol (LDL-C) < 160 mg/dl, and body mass index between 18 and 35 kg/m^2. Subjects having an LDL-C < 160 mg/dl while stabilized on atorvastatin 20 mg were eligible to participate in the atorvastatin arm of this study. The study protocols were approved by the Human Investigation Review Committee of each institution, and informed, written consent was obtained from each participant.

Experimental design

The study design was a single-blind, placebo-controlled, fixed-sequence study. A total of 19 subjects were enrolled, with 9 subjects studied on a background of atorvastatin treatment. A detailed description of the study design has been reported previously (1). Briefly, the study consisted of an introductory period of 2–4 weeks, during which time subjects were screened and, if necessary (LDL-C > 160 mg/dl), stabilized on atorvastatin 20 mg, which continued throughout the study. All subjects next received placebo for 4 weeks, followed by torcetrapib 120 mg once daily for an additional 4 weeks. Six subjects treated with torcetrapib alone also participated in a third phase, in which they received high-dose torcetrapib (120 mg twice daily) for an additional 4 weeks.

At the end of each 4 week phase, subjects underwent a primed-constant infusion of deuterated leucine under constantly fed conditions to determine the kinetics of apoE (2, 3). At 11 AM, [5,5,5-D_3]L-leucine (10 μmol/kg body weight) was injected, intravenously, as a bolus followed by a continuous infusion (10 μmol/kg body weight/h) over a 15 h period. Blood samples were collected at 0, 30, 35, and 45 min and 1, 1.5, 2, 3, 4, 6, 9, 10, 12, and 15 h after the bolus. VLDL, IDL, and LDL were isolated by sequential ultracentrifugation.

Quantitation and isolation of apolipoproteins

ApoB levels were measured on a Hitachi 911 autoanalyzer (Hitachi, Inc.) with an immunoturbidimetric assay, using reagents and calibrators from Wako Diagnostics (Wako Chemicals USA, Richmond, VA). ApoE in plasma and lipoprotein fractions was measured by ELISA as described previously (7). The apoE/apoB molar ratio was calculated from the VLDL apoE and apoB concentrations, assuming molecular masses of 34,000 and 512,000 Da for apoE and apoB-100, respectively. ApoB-100 and apoE were isolated from VLDL by SDS-PAGE using a Tris-glycine buffer system as described previously (8). ApoE genotypes were determined by polymerase chain reaction as described previously (9).

Sample hydrolysis and derivatization

ApoE bands were excised from polyvinylidene difluoride membranes and hydrolyzed in 12 N HCl at 110°C for 24 h. After evaporative removal of the HCl, the amino acids were converted to n-propyl ester, *N*-heptafluorobutyramide derivatives (10). The derivatized amino acids were extracted into cyclohexane (Sigma-Aldrich Co.) for isotopic enrichment determination.

Determination of isotopic enrichment

Samples were analyzed on an Agilent Technologies 6890/5973N gas chromatograph-mass spectrometer. Gas chromatographic separations were performed on a 30 m × 0.32 mm J&W Scientific DX-4 column. The temperature of the electronic pressure-controlled splitless injection port was 210°C. With an initial helium pressure of the injection port of 14.7 p.s.i., a constant helium carrier gas flow rate of 4.7 ml/min was maintained throughout the run. The oven temperature was programmed to increase from 50°C to 150°C at 20°C/min, increase to 250°C at 60°C/min, and hold at 250°C for 5 min. The ion source temperature was 200°C. Selected ion monitoring at *m/z* 349 (derivatized [H_3]-leucine − HF^-) and *m/z* 352 (derivatized [D_3]-leucine − HF^-) under ethane electron-capture negative chemical ionization conditions was used to determine the areas under the curve of each ion. Moles percent enrichment ([D_3]-leucine/leucine) for each sample was calculated from the areas under the curve and converted to a tracer-tracee ratio (percent) according to the following formula:

$$\text{tracer-tracee} = e(t)/e(i) - e(t)$$

where e(t) is the enrichment at time t and e(i) represents the isotopic abundance of the infusate determined by GC-MS.

Kinetic analysis

The kinetics of VLDL apoB-100 for the subjects in this study have been reported previously and are shown for comparison (3). Under the postprandial conditions of the study, the triglyceride rich-lipoprotein fraction ($d < 1.006$ g/ml) contains both chylomicrons and VLDL. Because the majority of apoE in the triglyceride-rich lipoprotein fraction is associated with VLDL (apoB-100-containing lipoproteins) (11), we refer to apoE in the triglyceride-rich lipoprotein fraction as VLDL apoE. The kinetics of VLDL apoE were assessed using a previously described multicompartmental model (12). The SAAM II program was used to fit the model to the observed tracer data using a weighted least-squares approach to determine the best fit. ApoE pool size in each fraction was computed as follows:

$$\begin{aligned}\text{pool size (PS) (mg)} = {} & \text{apoE concentration (mg/dl)} \\ & \times \text{plasma volume (0.45 dl/kg body weight)} \\ & \times \text{body weight (kg)}\end{aligned}$$

ApoE PR in each fraction was calculated using the following formula:

$$\begin{aligned}\text{PR (mg/kg/day)} = {} & \text{PS (mg/pool)} \times \text{body weight (kg}^{-1}\text{)} \\ & \times \text{fractional catabolic rate (FCR; pools/day)}\end{aligned}$$

One subject treated with torcetrapib alone who also participated in the high-dose (120 mg torcetrapib twice daily) phase of the

TABLE 1. Changes in plasma and VLDL apoE and apoB levels during the placebo and torcetrapib 120 mg once-daily treatment phases (n = 9)

Study Phase	Plasma ApoE	VLDL ApoE	VLDL ApoB
		mg/dl	
Placebo (n = 9)	6.1 (2.0)	4.4 (1.7)	9.6 (2.8)
Torcetrapib	4.9 (1.9)	2.9 (1.4)	6.8 (1.5)
Mean change (SD)	−1.2 (1.7)	−1.4 (1.7)	−2.8 (2.3)
Mean difference (%)	−18	−28	−23
P	0.03	0.02	0.003
Atorvastatin + placebo (n = 9)	3.8 (0.7)	2.0 (0.9)	6.9 (2.0)
Atorvastatin + torcetrapib	4.1 (1.3)	2.0 (0.8)	5.2 (1.5)
Mean change (SD)	0.3 (1.1)	−0.1 (0.7)	−1.7 (1.5)
Mean difference (%)	8	3	−22
P	0.79	0.40	0.005

ApoE, apolipoprotein E. Values are averages of fed samples collected during the kinetic study. Significance for differences between treatments was determined using a paired *t*-test.

study had unsatisfactory tracer enrichment data. The tracer data for this subject were not modeled, and all data for this subject were excluded from the kinetic data analysis.

Statistical analyses

Results are presented as means and (SD). Statistical tests were performed using the Intercooled Stata 8.2 for Windows program (StataCorp, Chicago, IL). A paired *t*-test was used to assess differences between placebo and once-daily treatment groups. Repeated-measures ANOVA was used to assess differences among treatment groups in subjects who participated in the high-dose treatment phase. If significance was detected, a paired *t*-test was used to determine any individual group differences post hoc. $P < 0.05$ was considered statistically significant. The percentage change with torcetrapib relative to placebo was computed for individual subjects.

RESULTS

Plasma lipids

Changes in plasma lipids and apoB in subjects treated with torcetrapib 120 mg once daily have been reported previously (1, 3). Briefly, this group consisted of nine males and one female. Eight subjects had the *APOE3/APOE3* genotype, whereas the remaining subjects were either *APOE2/APOE3* (n = 1) or *APOE3/APOE4* (n = 1) heterozygotes. After treatment with placebo, these subjects had mean total cholesterol, LDL-C, HDL-C, and triglyceride levels of 199 ± 26, 136 ± 24, 32 ± 7, and 154 ± 56 mg/dl, respectively, at the end of the placebo period. After treatment with torcetrapib 120 mg once daily, these values changed to 200 ± 36, 114 ± 40, 46 ± 14, and 109 ± 51 mg/dl, respectively. Plasma and VLDL apoE levels (**Table 1**) during the placebo period were 6.1 ± 2.0 and 4.4 ± 1.7 mg/dl, respectively. These declined to 4.9 ± 1.9 ($P = 0.03$) and 2.9 ± 1.4 ($P = 0.02$) mg/dl, respectively, after treatment with torcetrapib.

The subjects participating in the high-dose phase of the study (n = 6) had mean total cholesterol, LDL-C, HDL-C, and triglyceride levels of 199 ± 26, 136 ± 24, 34 ± 5, and 154 ± 56 mg/dl, respectively, at the end of the placebo period. After low-dose torcetrapib treatment (120 mg once daily), these values changed to 207 ± 45, 132 ± 38, 53 ± 12, and 135 ± 74 mg/dl, respectively. After high-dose torcetrapib treatment (120 mg twice daily), these values changed to 200 ± 36, 114 ± 40, 70 ± 15, and 109 ± 51 mg/dl, respectively. Plasma and VLDL apoE levels during the placebo period were 6.1 ± 2.2 and 4.2 ± 1.7 mg/dl, respectively. During treatment with 120 mg torcetrapib once daily, these values changed to 6.2 ± 2.2 and 5.1 ± 2.1 mg/dl, respectively, and on the high dose they changed to 5.3 ± 1.6 and 3.0 ± 1.0 mg/dl, respectively.

Changes in plasma lipids and apoB in the group treated with torcetrapib 120 mg once daily while on a background of atorvastatin 20 mg have been reported previously (1, 3). This group consisted of eight males and one female, of which seven subjects had the *APOE3/APOE3* genotype, whereas the remaining subjects were either *APOE2/APOE3* (n = 1) or *APOE3/APOE4* (n = 1) heterozygotes. These subjects had mean total cholesterol, LDL-C, HDL-C, and triglyceride levels of 150 ± 33, 94 ± 30, 29 ± 4, and 122 ± 47 mg/dl, respectively, after treatment with placebo (atorvastatin + placebo). Adding torcetrapib 120 mg once daily to the existing atorvastatin treatment (atorvastatin + torcetrapib) resulted in changes in the mean values to 141 ± 21, 76 ± 19, 47 ± 10, and 98 ± 42 mg/dl, respectively. Plasma and VLDL apoE levels (Table 1) during the atorvastatin + placebo treatment period were 3.8 ± 0.7 and 2.0 ± 0.9 mg/dl, respectively. During treatment with atorvastatin + torcetrapib, these changed to 4.1 ± 1.3 and 2.0 ± 0.8 mg/dl, respectively, neither of which was statistically significant.

ApoE kinetics

Subjects treated with torcetrapib 120 mg once daily had a significant decrease (−28%; $P = 0.01$) in VLDL apoE pool size compared with the placebo treatment period

TABLE 2. VLDL apoE and apoB-100 kinetic parameters from subjects who underwent treatment with torcetrapib 120 mg once daily (n = 9)

Study Phase	Plasma ApoE Pool Size	VLDL ApoE Pool Size	VLDL ApoB Pool Size	VLDL ApoE FCR	VLDL ApoB FCR	VLDL ApoE PR	VLDL ApoB PR
		mg		*pools/day*		*mg/kg/day*	
Placebo	232 (73)	167 (64)	370 (110)	3.8 (1.2)	6.2 (2.6)	7.2 (4.0)	24.1 (6.1)
Torcetrapib	187 (70)	113 (55)	264 (73)	5.8 (1.6)	9.4 (2.5)	7.7 (4.3)	27.6 (6.9)
Mean change (SD)	−46 (60)	−54 (59)	−106 (80)	2.1 (2.2)	3.2 (3.1)	0.5 (3.1)	3.5 (3.9)
Mean difference (%)	−18	−28	−23	77	67	14	15
P	0.03	0.01	0.002	0.01	0.007	0.32	0.01

FCR, fractional catabolic rate; PR, production rate. Significance for differences between treatments was determined using a paired *t*-test.

TABLE 3. VLDL apoB-100 and apoE kinetic parameters from subjects (n = 5) who underwent low-dose (120 mg once daily) followed by high-dose (120 mg twice daily) torcetrapib treatment

Study Phase	Plasma ApoE Pool Size	VLDL ApoE Pool Size	VLDL ApoB Pool Size	VLDL ApoE FCR	VLDL ApoB-100 FCR	VLDL ApoE PR	VLDL ApoB-100 PR
		mg		*pools/day*		*mg/kg/day*	
Placebo	218 (83)	150 (62)	326 (130)	4.0 (0.6)	6.8 (3.2)	7.2 (2.3)	24.0 (3.9)
Torcetrapib 120 mg once daily	179 (75)	99 (62)	227[a] (53)	5.7 (1.9)	10.4[a] (2.1)	6.8 (3.9)	28.7 (3.2)
Mean change (SD)	−39 (77)	−51 (77)	−100 (94)	1.8 (2.4)	3.6 (3.6)	−0.4 (3.4)	4.7 (2.6)
Mean difference versus placebo (%)	−15	−27	−20	54	71	−6	21
Torcetrapib 120 mg twice daily	190 (61)	110 (38)	224[a] (62)	6.4 (1.6)	9.7[a] (2.4)	8.5 (2.4)	23.3 (10.3)
Mean change (SD)	−27 (63)	−40 (46)	−102 (75)	2.4 (2.0)	2.9 (0.9)	1.3 (1.6)	−0.7 (9.7)
Mean difference versus placebo (%)	−6	−20	−26	68	51	23	−2
P (ANOVA)	0.43	0.16	0.02	0.07	0.04	0.50	0.38

Significant differences among treatments were determined using a repeated-measures ANOVA.
[a] This group is significantly different from placebo determined using a paired *t*-test post hoc.

(**Table 2**). This was associated with a significant increase (77%; $P = 0.01$) in the VLDL apoE FCR. These subjects also had a 14% increase in the VLDL apoE PR that was not statistically significant.

Subjects participating in the high-dose phase of the study had a 27% reduction in the VLDL apoE pool size at the 120 mg once-daily dose and a 20% reduction in the VLDL apoE pool size at the high dose (120 mg twice daily) of torcetrapib ($P = 0.16$) compared with placebo treatment (**Table 3**). The decrease in VLDL apoE pool size was associated with an increase in the VLDL apoE FCR, which increased by 54% at the 120 mg once-daily dose and by 68% at the high dose of torcetrapib, which was of borderline significance ($P = 0.07$). The VLDL apoE PR was reduced by 6% at the 120 mg once-daily dose but was increased by 23% at the high dose, both of which were nonsignificant changes ($P = 0.50$).

The addition of torcetrapib 120 mg once daily to existing atorvastatin treatment (atorvastatin + torcetrapib) resulted in no significant change in the VLDL apoE pool size (**Table 4**). This was associated with a significant increased the VLDL apoE FCR by 25% ($P = 0.03$) that was counteracted by a 21% increase in the VLDL apoE PR ($P = 0.03$). The net effect of these opposing changes in VLDL apoE FCR and PR accounted for the lack of change in the VLDL apoE pool size.

ApoE content of VLDL, IDL, and LDL

We examined the effect of torcetrapib treatment on the content of apoE in VLDL, IDL, and LDL by determining the molar ratio apoE/apoB in these fractions. Overall, VLDL had the greatest content of apoE (molar ratio ~ 4.3–7.4), followed by IDL (molar ratio ~ 0.7–1.0), with LDL having the lowest content of apoE (molar ratio ~ 0.02–0.08) (**Fig. 1**). Treatment with torcetrapib 120 mg once daily had no significant effect on the content of apoE in VLDL, IDL, or LDL (Fig. 1A). Similarly, treatment with high-dose (120 mg twice daily) torcetrapib also had no significant effect on the content of apoE in VLDL, IDL, or LDL (Fig. 1B). In contrast, subjects studied on a background of atorvastatin had a significant increase in the content of apoE in VLDL ($P = 0.01$) after torcetrapib (atorvastatin + torcetrapib) treatment (Fig. 1C). These subjects also had a significant reduction in the content of apoE in IDL in response to torcetrapib but no change in the content of apoE in LDL.

DISCUSSION

CETP mediates the net transport of cholesteryl ester from HDL to apoB-containing lipoproteins and, in turn, the net transfer of triglyceride from apoB-containing lipoproteins to HDL. Inhibition of CETP increases HDL-C by preventing HDL cholesteryl ester exchange with apoB-containing lipoproteins. The use of two CETP inhibitors, JTT-705 and torcetrapib, resulted in significant increases in HDL and significant reductions in plasma triglyceride and LDL-C levels (13, 14). All clinical development of torcetrapib was halted after the independent Data and Safety Monitoring Board monitoring the ILLUMINATE

TABLE 4. VLDL apoB-100 and apoE kinetic parameters from subjects who underwent torcetrapib 120 mg once-daily treatment on a background of atorvastatin (n = 9)

Study Phase	Plasma ApoE Pool Size	VLDL ApoE Pool Size	VLDL ApoB Pool Size	VLDL ApoE FCR	VLDL ApoB FCR	VLDL ApoE PR	VLDL ApoB PR
		mg		*pools/day*		*mg/kg/day*	
Atorvastatin + placebo	151 (34)	80 (38)	272 (81)	5.6 (1.7)	8.4 (4.9)	4.7 (1.6)	23.4 (6.3)
Atorvastatin + torcetrapib	159 (46)	77 (32)	201 (57)	6.7 (2.0)	11.6 (4.1)	5.7 (2.3)	25.5 (7.2)
Mean change (SD)	−8 (38)	−4 (26)	−71 (60)	1.1 (1.5)	3.1 (5.3)	1.0 (1.4)	2.1 (9.2)
Mean difference (%)	7	3	−22	25	64	21	16
P	0.27	0.35	0.004	0.03	0.06	0.03	0.26

Significance for differences between treatments was determined using a paired *t*-test.

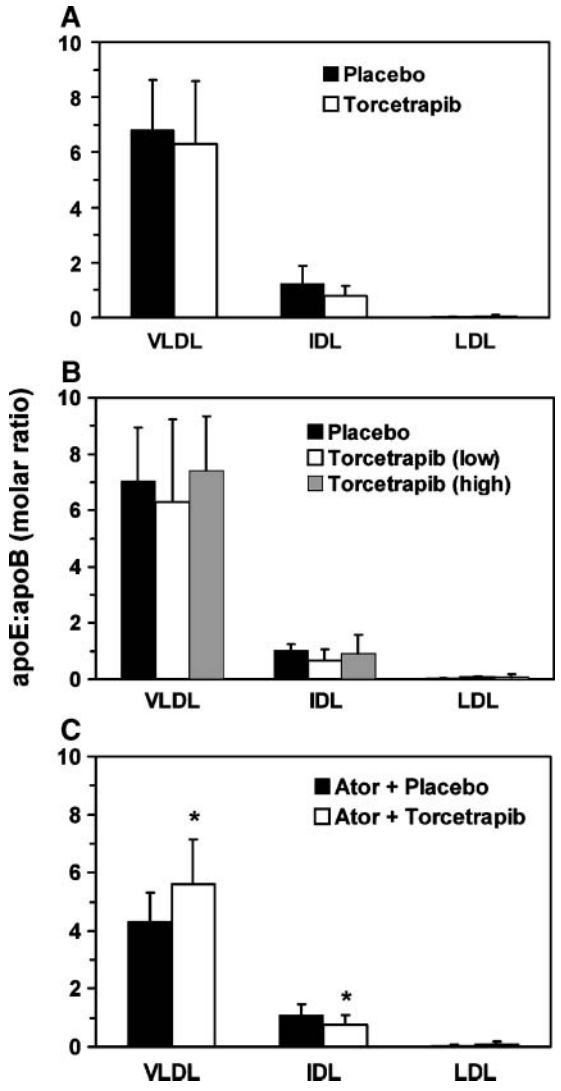

Fig. 1. Apolipoprotein E (apoE) content of VLDL, intermediate density lipoprotein (IDL), and LDL in response to cholesteryl ester transfer protein inhibition with torcetrapib. Results are shown for subjects treated with torcetrapib 120 mg once daily (n = 9) (A), subjects participating in the high-dose phase (n = 5) (B), and subjects treated with torcetrapib 120 mg once daily while maintained on a background of atorvastatin (Ator) 20 mg (n = 9) (C). The significance of changes between the placebo and torcetrapib 120 mg once-daily groups in A and C was determined using a paired *t*-test. Differences among treatments in B were tested using a repeated-measures ANOVA. Values shown are means ± SD. * $P < 0.05$ versus placebo.

morbidity and mortality study for torcetrapib/atorvastatin recommended terminating the study because of a statistically significant imbalance in all-cause mortality between subjects receiving torcetrapib/atorvastatin and those receiving atorvastatin alone (15). Full details of the cause of this imbalance have yet to be determined. Nevertheless, mechanistic studies conducted with torcetrapib provide insights into the effect of CETP and CETP inhibitors on lipoprotein metabolism.

In the current study with torcetrapib, we found that CETP inhibition significantly increased HDL-C and significantly reduced plasma concentrations of VLDL, IDL, LDL-C, and apoB. In subjects treated with torcetrapib alone, these reductions were associated with a decrease in the plasma and VLDL apoE concentrations, similar to the decrease that was observed for VLDL apoB-100. However, subjects who added torcetrapib to existing atorvastatin treatment had no change in plasma or VLDL apoE levels despite a reduction in the VLDL apoB-100 concentration.

The mechanism responsible for the decrease in the plasma apoE concentration in subjects treated with torcetrapib alone was enhanced clearance of VLDL apoE. Because apoE is considered an exchangeable apolipoprotein, it is possible that it could be cleared from plasma independently of apoB, a nonexchangeable constituent of VLDL. Our results are consistent with the idea that increased VLDL apoE clearance was attributable to enhanced whole VLDL particle clearance, because VLDL apoB clearance was also increased after CETP inhibition. Enhanced whole particle clearance of VLDL in response to torcetrapib could be a direct result of VLDL apoE acting as a ligand for hepatic lipoprotein receptors (4). Although there was no change in the amount of apoE in VLDL after torcetrapib treatment in subjects treated with torcetrapib alone, we previously reported that VLDL from these subjects is enriched with triglyceride after torcetrapib treatment (3). This could influence the conformation of apoE in VLDL and increase the affinity of apoE for lipoprotein receptors (16). Alternatively, triglyceride enrichment of VLDL may make VLDL a better substrate for lipoprotein lipase (17) or modify the content of other exchangeable proteins, such as apoC-II, apoC-III, or apoA-V, that influence the lipolysis of VLDL triglyceride, leading to enhanced VLDL clearance (18–20).

In contrast to what was observed after treatment with torcetrapib alone, adding torcetrapib to existing atorvastatin treatment resulted in no significant change in the VLDL apoE pool size. Although the VLDL apoE FCR was increased in response to torcetrapib in these subjects, likely as a result of enhanced whole VLDL particle clearance, because VLDL apoB clearance was also increased, this was counteracted by an increase in the VLDL apoE PR. The reason for the increase in the VLDL apoE PR may relate to the reduction in serum lathosterol concentrations in response to torcetrapib that we reported previously for these subjects (2). Reduced serum lathosterol, a marker of hepatic cholesterol synthesis, may indicate that enzymes in the cholesterol synthesis pathway are downregulated in response to increased cholesterol delivery to liver after CETP inhibition. ApoE transcription has been reported to be upregulated in response to increased hepatic cholesterol content (21), perhaps as a way of promoting excess free cholesterol transport away from the liver. Atorvastatin monotherapy has been reported to reduce apoE production (22), presumably in response to a decrease in the hepatic cholesterol content after inhibition of cholesterol synthesis. Treatment with torcetrapib may counteract the effect of atorvastatin on reducing apoE production by promoting

cholesterol delivery to liver via LDL and HDL, leading to increased hepatic cholesterol content and increased apoE production.

We reported previously that the apoE content of VLDL, measured as the molar ratio of apoE to apoB, was a significant predictor of the LDL apoB-100 PR, with an increased VLDL apoE content being associated with reduced LDL apoB-100 production (6). In the current study, we found that the VLDL apoE content was unchanged in subjects treated with torcetrapib alone but was increased significantly in subjects who added torcetrapib to existing atorvastatin treatment. In both instances, there was significantly less apoE present in IDL and LDL, consistent with the concept that apoE-poor VLDL is preferentially channeled toward IDL and LDL production (5, 23, 24). Subjects treated with torcetrapib alone had no significant change in the VLDL apoE content, which could explain the lack of change in the LDL apoB-100 PR in response to torcetrapib. However, subjects who added torcetrapib to existing atorvastatin treatment had a significant increase in VLDL apoE content, which likely contributed toward the observed reduction in the LDL apoB-100 PR (3).

In conclusion, our results provide new insights into the mechanisms responsible for the changes in the metabolism of apoE in response to CETP inhibition. These data indicate that treatment with the CETP inhibitor torcetrapib alone decreases the VLDL apoE pool size to a similar extent as the VLDL apoB pool size by increasing VLDL apoE clearance. The addition of torcetrapib to existing atorvastatin treatment had no significant effect on the VLDL apoE pool size, despite an increased VLDL apoE clearance caused by a concomitant increase in the VLDL apoE PR. These latter changes were associated with an increase in the VLDL apoE content that likely contributed to reduced LDL apoB-100 production in these subjects in response to torcetrapib.

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