

ORIGINAL ARTICLE

## Effects of an Inhibitor of Cholesteryl Ester Transfer Protein on HDL Cholesterol

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### ABSTRACT

#### BACKGROUND

Decreased high-density lipoprotein (HDL) cholesterol levels constitute a major risk factor for coronary heart disease; however, there are no therapies that substantially raise HDL cholesterol levels. Inhibition of cholesteryl ester transfer protein (CETP) has been proposed as a strategy to raise HDL cholesterol levels.

#### METHODS

We conducted a single-blind, placebo-controlled study to examine the effects of torcetrapib, a potent inhibitor of CETP, on plasma lipoprotein levels in 19 subjects with low levels of HDL cholesterol (<40 mg per deciliter [1.0 mmol per liter]), 9 of whom were also treated with 20 mg of atorvastatin daily. All the subjects received placebo for four weeks and then received 120 mg of torcetrapib daily for the following four weeks. Six of the subjects who did not receive atorvastatin also participated in a third phase, in which they received 120 mg of torcetrapib twice daily for four weeks.

#### RESULTS

Treatment with 120 mg of torcetrapib daily increased plasma concentrations of HDL cholesterol by 61 percent ( $P<0.001$ ) and 46 percent ( $P=0.001$ ) in the atorvastatin and non-atorvastatin cohorts, respectively, and treatment with 120 mg twice daily increased HDL cholesterol by 106 percent ( $P<0.001$ ). Torcetrapib also reduced low-density lipoprotein (LDL) cholesterol levels by 17 percent in the atorvastatin cohort ( $P=0.02$ ). Finally, torcetrapib significantly altered the distribution of cholesterol among HDL and LDL subclasses, resulting in increases in the mean particle size of HDL and LDL in each cohort.

#### CONCLUSIONS

In subjects with low HDL cholesterol levels, CETP inhibition with torcetrapib markedly increased HDL cholesterol levels and also decreased LDL cholesterol levels, both when administered as monotherapy and when administered in combination with a statin.

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**L**ARGE-SCALE CLINICAL TRIALS IN WHICH inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (statins) have been used to reduce low-density lipoprotein (LDL) cholesterol levels have shown marked improvements in clinical outcomes.<sup>1</sup> Despite the favorable effects of statins on the risk of coronary heart disease, many cardiovascular events are not prevented by statin therapy.<sup>2-5</sup> Hence, there is a great deal of interest in identifying therapies capable of further reducing the risk of coronary heart disease. One such potential therapeutic target is a low level of high-density lipoprotein (HDL) cholesterol. A low level of HDL cholesterol is the most common lipid abnormality observed in patients with known coronary heart disease; in about half of these patients this is the primary lipid abnormality.<sup>6</sup> Statins have only moderate effects on HDL cholesterol levels, raising them by 5 to 10 percent. Although fibrates and niacin can raise HDL cholesterol levels, the increases are rarely greater than 25 percent. The concept of therapies targeted toward HDL metabolism has gained support with the recent report of a small clinical trial in which five weekly infusions of apolipoprotein A-I Milano-phospholipid complexes induced regression of coronary atherosclerosis, as assessed by intravascular ultrasonography.<sup>7,8</sup>

Another HDL cholesterol-raising strategy actively being explored is the inhibition of cholesteryl ester transfer protein (CETP). CETP is a plasma glycoprotein that facilitates the transfer of cholesteryl esters from HDL cholesterol to apolipoprotein B-containing lipoproteins.<sup>9</sup> Humans with CETP deficiency due to molecular defects in the *CETP* gene have markedly elevated plasma levels of HDL cholesterol and apolipoprotein A-I,<sup>10-12</sup> leading to the concept that CETP inhibition might increase HDL cholesterol levels. In animal models, inhibition of CETP by monoclonal antibodies,<sup>13,14</sup> antisense oligonucleotides,<sup>15,16</sup> small molecules,<sup>17,18</sup> or vaccine-induced antibodies<sup>19</sup> has resulted in increased HDL cholesterol levels. In addition, a small-molecule inhibitor of CETP has been shown to increase HDL cholesterol levels to a moderate extent in healthy persons with normal HDL cholesterol levels.<sup>20</sup>

The current study was designed to examine the effects of a novel CETP inhibitor, torcetrapib, on plasma lipoproteins in patients with a low level of HDL cholesterol (<40 mg per deciliter [1.0 mmol per liter]) when given either alone or in combination with atorvastatin.

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## METHODS

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### SUBJECTS

The subjects in this investigator-initiated study were recruited at the New England Medical Center, Boston, and the University of Pennsylvania School of Medicine, Philadelphia. Patients were eligible to participate if they met the following criteria: an age between 18 and 70 years, an HDL cholesterol level below 40 mg per deciliter, a triglyceride level below 400 mg per deciliter (4.5 mmol per liter), an LDL cholesterol level of 160 mg per deciliter (4.1 mmol per liter) or less and a body-mass index (the weight in kilograms divided by the square of the height in meters) between 18 and 35. Subjects who had an LDL cholesterol level above 160 mg per deciliter were considered for participation if they met all the inclusion criteria, including an LDL cholesterol level of 160 mg per deciliter or less, once treatment with 20 mg of atorvastatin daily had been started and their plasma lipid levels had stabilized, a step requiring at least two weeks.

Exclusion criteria included the following characteristics: childbearing potential; regular consumption of more than two units of alcohol per week (where one unit consists of 12 oz (360 ml) of beer, 5 oz (150 ml) of wine, or 1 oz (30 ml) of liquor); cigarette smoking; treatment within the previous six weeks with any medication, other than a statin, that is known to affect plasma lipid levels; evidence of renal or endocrine disease (including diabetes) or a strong family history of renal or endocrine disease; a history of hepatic disease or substantial elevations in liver-enzyme levels at the time of screening; congestive heart failure, unstable angina, or myocardial infarction within the previous six months; a clinically relevant electrocardiographic abnormality at the time of screening; and evidence on a urinary screen of illegal drug use.

The study protocol was approved by the human-investigation review committee at each center, and written informed consent was obtained from all the participants. The investigators had complete access to the primary data and conducted the data analysis, which was independently confirmed by investigators at the sponsoring institution.

### STUDY DESIGN AND PROTOCOL

This was a single-blind, placebo-controlled, fixed-sequence study designed to examine the effects of torcetrapib on plasma lipoproteins and lipoprotein

metabolism in subjects with low HDL cholesterol levels. A total of 19 subjects were enrolled; 9 received atorvastatin, and 10 did not. The study began with an introductory period lasting two to four weeks, during which the subjects were screened and, if their LDL cholesterol level was above 160 mg per deciliter, stabilization with 20 mg of atorvastatin daily had been achieved. All the subjects next received placebo for four weeks, followed by 120 mg of torcetrapib daily for an additional four weeks; a subgroup of the subjects not taking atorvastatin went on to receive 120 mg of torcetrapib twice daily for the following four weeks.

All the subjects underwent a safety evaluation at a clinic visit four weeks after the completion of torcetrapib administration. Blood samples were obtained for safety testing and the measurement of plasma lipid, lipoprotein, and apolipoprotein levels at screening and at weeks 4, 8, 12, and 16, as well as at week 20 in the subgroup that received 120 mg of torcetrapib twice daily.

#### BIOCHEMICAL ANALYSIS

Blood samples were collected from the subjects after a 12-to-14-hour fast in tubes containing 0.1 percent EDTA. Plasma was isolated by centrifugation at 2500 rpm at 4°C for 20 minutes. Plasma levels of total cholesterol and triglycerides were measured by enzymatic methods, as previously described.<sup>21</sup> The levels of unesterified cholesterol and phospholipid were determined with an AutoAnalyzer (Hitachi 911) and reagent kits (Wako Diagnostics). Plasma levels of esterified cholesterol were calculated by subtracting the unesterified cholesterol level from the total cholesterol level. Plasma LDL cholesterol levels were measured directly with the use of a reagent kit (Genzyme Diagnostics).<sup>22</sup> HDL cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins,<sup>23</sup> and the cholesterol content of HDL<sub>3</sub> (a subclass of HDL cholesterol) was assessed after differential polyanion precipitation.<sup>24</sup> Plasma levels of apolipoproteins A-I, A-II, and B were measured on an AutoAnalyzer (Cobas Fara II, Roche) with immunoturbidimetric assays (Wako Diagnostics reagents and calibrators). CETP inhibition in human plasma samples was determined by measuring the transfer of <sup>3</sup>H-cholesteryl oleate from HDL to apolipoprotein B-containing lipoproteins and the transfer of <sup>14</sup>C-cholesteryl oleate from

LDL to HDL. Plasma samples assayed for CETP activity were those collected immediately before the administration of each successive dose of torcetrapib (i.e., at trough), 24 and 12 hours after the last administered dose in the subjects who received once-daily doses and those who received twice-daily doses, respectively.

#### NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Lipoprotein subclass concentrations were determined by proton nuclear magnetic resonance spectroscopy, as previously described.<sup>25</sup> For purposes of the current study, subclasses of LDL and HDL were defined as follows: large LDL, 21 to 23 nm; small LDL, 18 to 20 nm; large HDL, 8.8 to 13.0 nm; and small HDL, 7.3 to 7.7 nm.

#### STATISTICAL ANALYSIS

Because of the large number of end points examined, a uniform method of analysis for all the end points was used. In addition to visual examination of histograms and box plots, the Shapiro-Wilk goodness-of-fit test was used to assess the normality of end-point data.<sup>26</sup> No significant departures from normality were detected, with the exception of triglyceride levels, which were log-transformed before analysis. Paired t-tests were used to assess differences between the placebo and drug phases within each group, whereas two-sample t-tests were used to detect statistically significant differences between the subjects who received atorvastatin and those who did not (SPSS software, version 10.0). The percentage change with torcetrapib relative to placebo was computed for individual subjects and summarized descriptively for each group of subjects. The data are presented as means  $\pm$ SD.

## RESULTS

#### CHARACTERISTICS OF THE SUBJECTS

The characteristics of the study subjects at the time of screening are provided in Table 1. A total of 19 subjects (17 men and 2 women) were enrolled in and completed the trial. The subjects who received atorvastatin and those who did not were similar with respect to age, sex distribution, and levels of HDL cholesterol and apolipoprotein A-I. As expected, the levels of LDL cholesterol and apolipoprotein B were significantly lower in the atorvastatin cohort.

**Table 1. Characteristics of the Subjects at Randomization.\***

Variable	Atorvastatin plus Torcetrapib (120 mg/day) (N=9)	Torcetrapib Alone (120 mg/day)† (N=10)‡
Age (yr)	51±10	49±13
Sex (M/F)	8/1	9/1
Body-mass index	28.9±3.2	27.9±2.5
Cholesterol (mg/dl)		
Total	152±20‡	203±32
LDL	90±24‡	134±25
HDL	32±7	33±6
Ratio of total cholesterol to HDL cholesterol	4.9±1.2§	6.5±1.5
Triglycerides (mg/dl)	145±94	162±89
Apolipoprotein (mg/dl)		
A-I	114±19	117±8
A-II	33±4	31±3
B	87±13¶	104±11

\* Plus-minus values are means ±SD. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.

† Six of the 10 subjects who received only torcetrapib went on to receive 120 mg of torcetrapib twice daily for an additional four weeks. The characteristics of these six subjects did not differ significantly from those of the entire group.

‡ P<0.001 for the comparison with the subjects who did not receive atorvastatin.

§ P=0.04 for the comparison with the subjects who did not receive atorvastatin.

¶ P=0.01 for the comparison with the subjects who did not receive atorvastatin.

#### SAFETY AND ADVERSE EVENTS

Torcetrapib, alone or in combination with atorvastatin, resulted in no clinically significant changes in vital signs, serum chemical values, or hematologic values. There were no serious adverse events and no withdrawals due to adverse events. The 19 subjects reported a total of 28 adverse events during the study, 20 of which were mild and 8 of which were moderate. Seventeen of the adverse events, reported by 13 patients, were considered to be treatment related; 14 were mild and 3 were moderate. Four adverse events occurred in three subjects who had received atorvastatin: one had a headache while receiving placebo, two had a headache while receiving 120 mg of torcetrapib daily, and one of the two latter patients also had pain. Thirteen adverse events occurred among the subjects who did not receive atorvastatin: one had dizziness while receiving placebo; six had a headache, asthenia, pain (in two subjects), dyspepsia, herpes simplex, herpes zoster, or

sweating while receiving 120 mg of torcetrapib daily; and three had headache, dyspepsia, amnesia, or abnormal thinking while receiving 120 mg of torcetrapib twice daily.

#### EFFECTS ON PLASMA LEVELS OF HDL CHOLESTEROL AND APOLIPOPROTEINS A-I AND A-II

Treatment with 120 mg of torcetrapib daily resulted in trough CETP-activity values that were lower than the values measured during placebo administration: the reductions were 38±22 percent (P=0.001) among the subjects who received atorvastatin and 28±16 percent (P=0.003) among those who did not. Among the subjects who also received 120 mg of torcetrapib twice daily, the trough CETP-activity values were 65±16 percent lower than the values measured during the placebo phase (P=0.01). Torcetrapib had striking effects on plasma HDL cholesterol levels (Table 2). At a dose of 120 mg daily, it increased plasma HDL cholesterol levels by 61 percent, from 29±4 mg per deciliter (0.8±0.1 mmol per liter) to 47±10 mg per deciliter (1.2±0.3 mmol per liter), among the subjects who received atorvastatin (P<0.001) and by 46 percent, from 32±7 mg per deciliter (0.8±0.2 mmol per liter) to 46±14 mg per deciliter (1.2±0.4 mmol per liter), among those who did not receive atorvastatin (P=0.001). Among those who went on to receive 120 mg of torcetrapib twice daily, an increase in HDL cholesterol of 106 percent, from 34±5 mg per deciliter (0.9±0.1 mmol per liter) during the placebo phase to 70±15 mg per deciliter (1.8±0.4 mmol per liter), was observed (P<0.001).

Plasma levels of the HDL apolipoproteins A-I and A-II were also significantly increased by torcetrapib. Relative to the levels measured during placebo administration, the apolipoprotein A-I and A-II levels increased by 13 percent (P=0.003) and 10 percent (P<0.001), respectively, among the subjects who received 120 mg daily with atorvastatin, by 16 percent (P<0.001) and 12 percent (P=0.01) among those who received 120 mg daily without atorvastatin, and by 36 percent (P<0.001) and 21 percent (P<0.001) among those who received 120 mg twice daily.

#### EFFECTS OF TORCETRAPIB ON LIPIDS, LDL CHOLESTEROL, AND APOLIPOPROTEIN B

As shown in Table 3, torcetrapib had minimal effects on the plasma levels of cholesterol and phos-

**Table 2. Plasma HDL Cholesterol and Apolipoprotein A-I and A-II Levels at the End of the Placebo and Drug Phases.\***

Variable and Study Phase	Atorvastatin plus Torcetrapib (120 mg/day) (N=9)	Torcetrapib Alone (120 mg/day) (N=10)	Torcetrapib Alone (120 mg twice/day) (N=6)
<b>HDL cholesterol</b>			
Study phase (mg/dl)			
Placebo	29±4	32±7	34±5
Torcetrapib	47±10†	46±14‡	70±15†
Percentage change	61	46	106
<b>HDL<sub>2</sub> cholesterol</b>			
Study phase (mg/dl)			
Placebo	2.9±2.6	6.4±3.8	7.6±3.2
Torcetrapib	11.0±4.3†	11.1±7.8§	29.3±13.6¶
Percentage change	323	87	283
<b>HDL<sub>3</sub> cholesterol</b>			
Study phase (mg/dl)			
Placebo	26.2±4.8	25.2±3.6	26.2±2.5
Torcetrapib	35.9±9.9	32.6±6.5**	40.7±6.1
Percentage change	36	29	56
<b>Ratio of total cholesterol to HDL cholesterol</b>			
Study phase			
Placebo	5.3±1.4	6.4±1.6	6.0±1.4
Torcetrapib	3.1±0.6††	4.4±1.5†	3.0±1.0†
Percentage change	-40	-31	-51
<b>Apolipoprotein A-I</b>			
Study phase (mg/dl)			
Placebo	106±14	110±11	112±13
Torcetrapib	120±23**	127±15†	151±6†
Percentage change	13	16	36
<b>Apolipoprotein A-II</b>			
Study phase (mg/dl)			
Placebo	30±4	29±2	30±1
Torcetrapib	33±4†	33±5‡	36±3†
Percentage change	10	12	21

\* Plus-minus values are means ±SD. Minus signs denote a decrease. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. HDL denotes high-density lipoprotein.

† P<0.001 for the comparison with placebo.

‡ P=0.001 for the comparison with placebo.

§ P=0.02 for the comparison with placebo.

¶ P=0.004 for the comparison with placebo.

|| P=0.002 for the comparison with placebo.

\*\* P=0.003 for the comparison with placebo.

†† P=0.02 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

‡‡ P=0.01 for the comparison with placebo.

pholipids. Relative to placebo, torcetrapib at a dose of 120 mg daily in combination with atorvastatin reduced triglyceride levels by 18 percent (P=0.05); the reduction in the subjects who received 120 mg of torcetrapib twice daily was 26 percent (P=0.05).

Torcetrapib also reduced plasma levels of LDL cholesterol and apolipoprotein B. Among the subjects who received atorvastatin, torcetrapib reduced LDL cholesterol levels by 17 percent relative to placebo (P=0.02) and reduced apolipoprotein B levels

**Table 3. Plasma Levels of Lipids, LDL Cholesterol, and Apolipoprotein B at the End of the Placebo and Drug Phases.\***

Variable and Study Phase	Atorvastatin plus Torcetrapib (120 mg/day) (N=9)	Torcetrapib Alone (120 mg/day) (N=10)	Torcetrapib Alone (120 mg twice/day) (N=6)
<b>Total cholesterol</b>			
Study phase (mg/dl)			
Placebo	150±33†	192±28	199±26
Torcetrapib	141±21‡	193±42	200±36
Percentage change	-5	<1	<1
<b>Unesterified cholesterol</b>			
Study phase (mg/dl)			
Placebo	40±8‡	51±7	53±8
Torcetrapib	38±5§	52±11	53±9
Percentage change	-3	<1	-1
<b>Esterified cholesterol</b>			
Study phase (mg/dl)			
Placebo	111±26†	141±23	145±19
Torcetrapib	103±16‡	141±31	148±28
Percentage change	-5	0	2
<b>Triglycerides</b>			
Study phase (mg/dl)			
Placebo	122±47	161±58	154±56
Torcetrapib	98±42¶	154±67	109±51¶
Percentage change	-18	1	-26
<b>Phospholipids</b>			
Study phase (mg/dl)			
Placebo	169±20§	204±23	212±24
Torcetrapib	172±21**	215±38	226±26¶
Percentage change	2	4	7
<b>LDL cholesterol</b>			
Study phase (mg/dl)			
Placebo	94±30**	129±25	136±24
Torcetrapib	76±19††‡‡	119±36	114±40
Percentage change	-17	-8	-17
<b>Apolipoprotein B</b>			
Study phase (mg/dl)			
Placebo	86±15**	102±11	104±13
Torcetrapib	73±11§§	92±13¶¶	87±17††
Percentage change	-14	-10	-17

\* Plus-minus values are means ±SD. Minus signs denote a decrease. Because many secondary end points were analyzed, a P value of 0.045 may not be definitive. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. LDL denotes low-density lipoprotein.

† P=0.008 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

‡ P=0.004 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

§ P=0.003 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

¶ P=0.05 for the comparison with placebo.

|| P=0.05 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

\*\* P=0.02 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

†† P=0.02 for the comparison with placebo.

‡‡ P=0.006 for the comparison with 120 mg of torcetrapib daily among the subjects who did not receive atorvastatin.

§§ P=0.002 for the comparison with placebo.

¶¶ P=0.004 for the comparison with placebo.

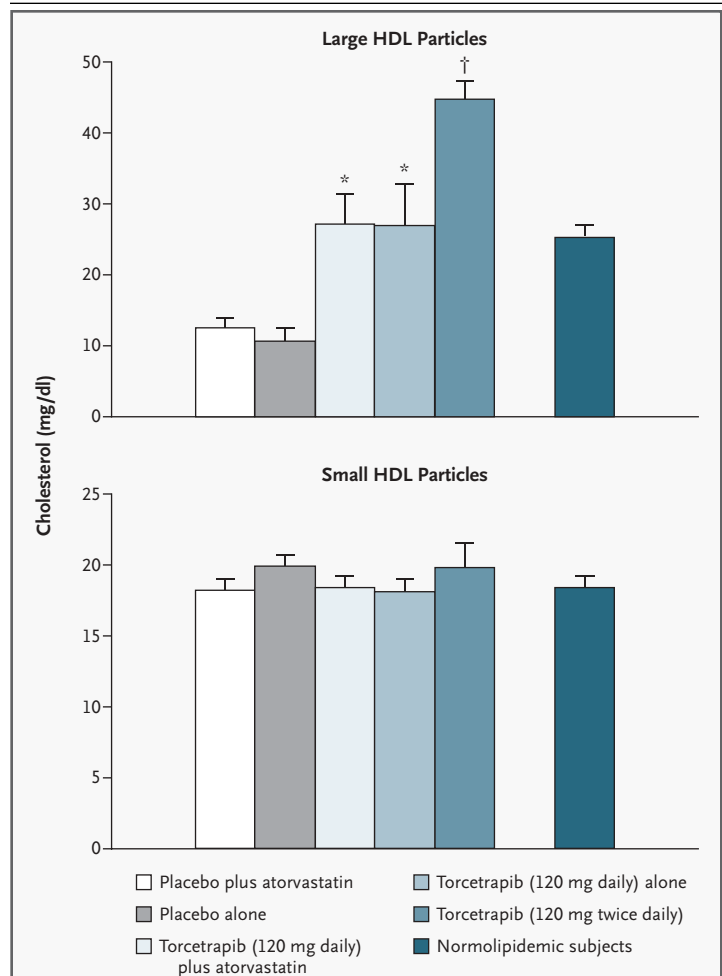
by 14 percent ( $P=0.002$ ). The corresponding reductions among those who did not receive atorvastatin were 8 percent ( $P$  not significant) and 10 percent ( $P=0.004$ ), respectively. Torcetrapib given at a dose of 120 mg twice daily was associated with nonsignificant reductions in the levels of LDL cholesterol (17 percent) and apolipoprotein B (17 percent) relative to the levels measured during the placebo phase.

**EFFECTS OF TORCETRAPIB ON HDL AND LDL SUBCLASSES**

Torcetrapib, as compared with placebo, had marked effects on the lipoprotein subclasses. The HDL<sub>2</sub> cholesterol level increased by 323 percent ( $P<0.001$ ) among the subjects who received atorvastatin and by 87 percent ( $P=0.02$ ) among those who did not. Among those who went on to receive 120 mg of torcetrapib twice daily, the HDL<sub>2</sub> cholesterol level increased by 283 percent over the level measured during the placebo phase ( $P=0.004$ ). HDL<sub>3</sub> cholesterol levels were, likewise, increased by torcetrapib but to a far lesser extent than were the HDL<sub>2</sub> cholesterol levels. Torcetrapib increased HDL<sub>3</sub> cholesterol levels by 36 percent ( $P=0.002$ ) among those who received atorvastatin and, among those who did not receive atorvastatin, by 29 percent ( $P=0.003$ ) and 56 percent ( $P=0.002$ ) at a dose of 120 mg once daily and a dose of 120 mg twice daily, respectively.

Cholesterol levels within large HDL particles, as assessed by nuclear magnetic resonance spectroscopy, increased by 133 percent over the values measured during the placebo phase ( $P=0.001$ ) among the subjects who received atorvastatin and by 199 percent among those who did not receive atorvastatin ( $P=0.001$ ) while receiving torcetrapib at a dose of 120 mg daily; the increase was 446 percent at a dose of 120 mg twice daily (Fig. 1). The changes in HDL subclass distribution translated into significant increases in the mean diameter of HDL particles among those who received 120 mg daily with atorvastatin (from  $8.5\pm 0.20$  nm to  $9.1\pm 0.33$  nm,  $P=0.002$ ), as well as among those who received 120 mg daily without atorvastatin (from  $8.4\pm 0.40$  nm to  $9.1\pm 0.65$  nm,  $P=0.002$ ); among those who received torcetrapib at a dose of 120 mg twice daily, the diameter increased from  $8.4\pm 0.43$  nm to  $9.7\pm 0.65$  nm ( $P<0.001$ ).

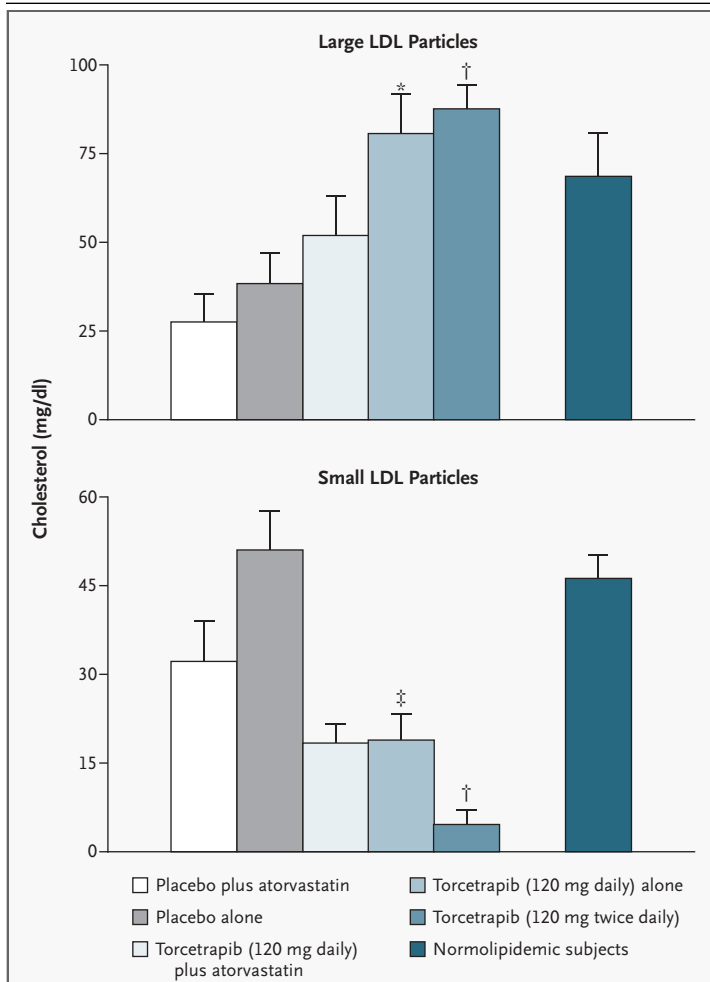
Torcetrapib also influenced LDL subclass distribution (Fig. 2). Without atorvastatin, torcetrapib at



**Figure 1. Mean ( $\pm$ SE) Levels of High-Density Lipoprotein (HDL) Subclasses in Each Group of Subjects during the Placebo and Torcetrapib Phases of the Study.**

All the subjects had low HDL cholesterol levels at base line. Data for a group of 38 age- and sex-matched subjects with normolipidemia are also provided.<sup>27</sup> As compared with placebo, torcetrapib significantly increased the levels of large HDL particles in each group (top panel); the dose of 120 mg daily normalized the levels of these particles. The asterisks ( $P=0.001$ ) and dagger ( $P<0.001$ ) indicate a significant difference from placebo. Torcetrapib did not significantly affect the levels of small HDL particles (bottom panel). To convert the values for cholesterol to millimoles per liter, multiply by 0.02586.

both doses (120 mg once daily and 120 mg twice daily) increased the levels of large LDL particles, by 257 percent ( $P=0.005$ ) and 294 percent ( $P=0.03$ ), respectively, relative to placebo. Conversely, the levels of small LDL cholesterol decreased with torcet-



**Figure 2.** Mean ( $\pm$ SE) Levels of Low-Density Lipoprotein (LDL) Subclasses in Each Group of Subjects during the Placebo and Torcetrapib Phases of the Study.

All the subjects had low HDL cholesterol levels at base line. Data for a group of 38 age- and sex-matched subjects with normolipidemia are also provided.<sup>27</sup> As compared with placebo, torcetrapib increased the levels of large LDL particles in each group (top panel). Conversely, the levels of small LDL particles were reduced by torcetrapib (bottom panel), with each of the study groups having a level lower than that in the group of subjects with normolipidemia ( $46\pm 49$  mg per deciliter [ $1.3\pm 1.3$  mmol per liter]). The asterisk ( $P=0.005$ ), daggers ( $P=0.03$ ), and double dagger ( $P=0.04$ ) indicate a significant difference from placebo. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586.

rapib — by 73 percent at a dose of 120 mg daily without atorvastatin ( $P=0.04$ ) and by 93 percent at a dose of 120 mg twice daily ( $P=0.03$ ). Torcetrapib did not significantly alter LDL subclasses in the subjects who received atorvastatin. The changes in LDL-

subclass distribution translated into significant increases in the mean diameter of LDL particles at both doses of torcetrapib without atorvastatin, from  $20.4\pm 0.89$  nm to  $21.4\pm 0.79$  nm at a dose of 120 mg daily ( $P=0.002$ ) and from  $20.4\pm 0.82$  nm to  $21.9\pm 0.15$  nm at a dose of 120 mg twice daily ( $P=0.003$ ).

## DISCUSSION

In the guidelines set forth by the third Adult Treatment Panel of the National Cholesterol Education Program,<sup>28</sup> a low HDL cholesterol level is defined categorically as a level below 40 mg per deciliter. The results of clinical trials indicate that even small increases in the HDL cholesterol level can significantly reduce the risk of coronary heart disease.<sup>29,30</sup> Primarily on the basis of epidemiologic data, Gordon et al. have reported that an increase in HDL cholesterol by 1 mg per deciliter is associated with a 2 to 4 percent reduction in the risk of cardiovascular events.<sup>31</sup> However, there remains a lack of well-tolerated drugs with clinically significant HDL-raising potential.

The current study was designed to examine the effects of a novel CETP inhibitor, torcetrapib, on plasma lipoproteins in patients with a low HDL cholesterol level ( $<40$  mg per deciliter), when given alone or in combination with 20 mg of atorvastatin. Torcetrapib significantly reduced CETP activity, measured at trough, in all subjects. This, in turn, led to marked increases in plasma HDL cholesterol levels, with both doses of torcetrapib (120 mg once daily and 120 mg twice daily) increasing HDL cholesterol from an average of  $30\pm 6$  mg per deciliter ( $0.7\pm 0.2$  mmol per liter) at base line to  $47\pm 10$  and  $70\pm 15$  mg per deciliter, respectively. These values represent approximately the 60th and 99th percentiles for men 50 to 54 years old.<sup>32</sup> Because the majority of patients who are candidates for CETP inhibition are also likely to be candidates for statin therapy, it is important to note that torcetrapib raised HDL cholesterol levels effectively in the subjects who also received atorvastatin.

Torcetrapib also reduced LDL cholesterol and apolipoprotein B levels. This finding is consistent with the fact that patients with homozygous defects in the *CETP* gene have reduced levels of LDL cholesterol.<sup>10-12</sup> The metabolic basis for the low levels of LDL cholesterol in persons with CETP deficiency is an increased rate of clearance of LDL cholesterol

from the plasma,<sup>33</sup> suggesting that the LDL-receptor pathway may be up-regulated in CETP deficiency. Of note, torcetrapib reduced LDL cholesterol levels to a further extent in the subjects who received atorvastatin in this study.

Patients with coronary heart disease have lower levels of large HDL particles<sup>27</sup> and higher levels of small, dense LDL particles<sup>27,34,35</sup> than do subjects without coronary heart disease. In the current study, concentrations of large HDL particles were significantly elevated by treatment with 120 mg of torcetrapib daily, to a value similar to that seen in age- and sex-matched subjects with normolipidemia.<sup>27</sup> This dose of torcetrapib also markedly reduced the levels of small, dense LDL particles both in subjects who had received atorvastatin and those who had not. Thus, the mean particle size of both HDL and LDL was significantly increased by torcetrapib. This result is interesting in the light of the recent report that a *CETP* polymorphism (replacement of isoleucine at position 405 with valine) that is linked to reduced CETP activity is significantly associated with longevity and large HDL and LDL particle size.<sup>36</sup>

Recently, the effects of another CETP inhibitor, JTT-705, on plasma lipid levels in humans were reported.<sup>20</sup> As reported, slightly hyperlipidemic subjects with normal HDL cholesterol levels were randomly assigned to placebo or daily treatment with 300, 600, or 900 mg of JTT-705. Increases in HDL cholesterol of 16 percent, 25 percent, and 34 percent, respectively, were observed in the three JTT-705 groups. A decrease in LDL cholesterol (by 7 percent) was seen only in the 900-mg group. In contrast, we report the effects of pharmacologic inhibition of CETP on plasma lipids and lipoproteins in patients with low HDL cholesterol levels, including some who also took a statin. Torcetrapib at a dose of 120 mg daily increased HDL cholesterol levels substantially more than did 900 mg of JTT-705, and torcetrapib at a dose of 120 mg twice daily raised HDL cholesterol levels to a level three times as high as did JTT-705. Moreover, the reduction in LDL cholesterol achieved with torcetrapib was more than twice that seen previously with 900 mg of JTT-705.

The relation of CETP activity to the risk of coronary heart disease remains controversial.<sup>37</sup> It is not

clear whether CETP-deficient persons are protected from coronary heart disease; they may even be at increased risk.<sup>38</sup> In the Honolulu Heart Program, a subgroup of persons heterozygous for a functional *CETP* mutation who had HDL cholesterol levels in the range of 40 to 60 mg per deciliter (1.0 to 1.6 mmol per liter) appeared to be at increased risk for coronary heart disease.<sup>38</sup> However, a recent analysis of seven-year prospective data from this study did not reveal a significant relation between heterozygosity for *CETP* mutations and coronary heart disease or stroke.<sup>37</sup> At the population level, it has been reported that a common *CETP* genetic variant (*TaqI* B2) is associated with reduced CETP activity, increased HDL cholesterol levels, and a reduced risk of coronary heart disease.<sup>39,40</sup> Moreover, inhibition of CETP in rabbits has been found to result in reduced atherosclerosis.<sup>16,17,19</sup>

In conclusion, torcetrapib is a well-tolerated and effective CETP inhibitor that has pronounced effects on plasma lipoproteins in patients with low HDL cholesterol levels. Torcetrapib not only increased the levels of HDL cholesterol and apolipoprotein A-I, it also reduced the levels of LDL cholesterol and apolipoprotein B, both when given as monotherapy and when given in combination with atorvastatin. Ultimately, the question of whether CETP inhibition is effective in reducing atherosclerotic cardiovascular disease in humans will be resolved only by trials based on hard clinical end points.

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