Association of Circulating Cholesteryl Ester Transfer Protein Activity With Incidence of Cardiovascular Disease in the Community

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Background—Plasma high-density lipoprotein cholesterol concentration is related inversely to the risk of cardiovascular disease (CVD). Inhibiting cholesteryl ester transfer protein (CETP) activity raises high-density lipoprotein cholesterol and may be cardioprotective, but an initial clinical trial with a CETP inhibitor was stopped prematurely because of increased CVD in treated patients, raising concerns about this approach. Data relating circulating CETP concentrations to CVD incidence in the community are conflicting.

Methods and Results—Plasma CETP activity was measured in 1978 Framingham Heart Study participants (mean age, 51 years; 54% women) who attended a routine examination in 1987–1990 and were free of CVD. On follow-up (mean, 15.1 years), 320 participants experienced a first CVD event (fatal or nonfatal coronary heart disease, cerebrovascular disease, peripheral vascular disease, or heart failure). In multivariable analyses adjusted for standard risk factors including high-density lipoprotein cholesterol, plasma CETP activity was related inversely to the incidence of CVD events (hazard ratio for activity, at or above the median of 0.72; 95% confidence interval, 0.57 to 0.90; \( P=0.004 \) [compared with below median]; hazard ratio per SD increment, 0.86; 95% confidence interval, 0.76 to 0.97; \( P=0.01 \)). The inverse association of CETP activity with CVD incidence remained robust in time-dependent models updating standard risk factors every 4 years and was maintained in analyses of incident “hard” CVD events (myocardial infarction, stroke, or heart failure).

Conclusions—In our prospective investigation of a community-based sample, lower plasma CETP activity was associated with greater CVD risk. These observations, if confirmed, challenge the concept that CETP inhibition may lower CVD risk. (Circulation. 2009;120:2414-2420.)

Key Words: epidemiology ■ lipids ■ risk factors
ated with torcetrapib treatment include “off-target” blood pressure—raising effects, induction of dysfunctional HDL by this agent, and/or a potential direct atherogenic effect of CETP inhibition.9–11 Studies also failed to demonstrate a reduction in progression of atherosclerosis in the carotid and coronary arteries with torcetrapib treatment despite impressive increases in HDL-C levels.12–14

Some genetic investigations have also raised questions about the desirability of inhibiting CETP. Although a recent meta-analysis noted a weak inverse association of common CETP gene variants (associated with lower CETP levels) with CVD risk,15 an updated meta-analysis separated community-based investigations from studies of high-risk samples and noted a direct association of CETP variants associated with lower CETP levels and higher HDL-C with CVD risk in the population-based samples.16 Some other studies focusing on partial genetic deficiency states have noted a paradoxical increased CVD risk in the setting of lower CETP activity.17–21

In addition, other investigations relating circulating CETP levels to CVD incidence in the community have yielded conflicting results. Thus, 2 recent reports indicated that lower blood CETP concentrations were associated with a greater risk of CVD in patients with prior coronary artery disease22 and in men with low triglyceride levels.23 In contrast, a case-control investigation from the European Prospective Investigation Into Cancer and Nutrition (EPIC)—Norfolk Study reported a positive association between plasma CETP levels and CVD that was confined to individuals with high triglyceride levels.24 These investigations were limited by the modest number of CVD events in some reports22,23 and by the case-control design in others.23,24 Accordingly, we related plasma CETP activity to the incidence of CVD prospectively in the community-based Framingham Heart Study.

Methods

Study Sample

The design of the Framingham Offspring Study has been published elsewhere.25 Participants in this cohort are seen at the Heart Study clinic approximately every 4 years. At each examination cycle, participants undergo laboratory testing for CVD risk factors, anthropometry, and a standardized medical history and physical examination targeted at surveillance for the incidence of CVD events. A team consisting of 3 physicians reviews all relevant medical information, hospitalization records, and physician office visits to adjudicate CVD events using standardized criteria.31 A separate group consisting of neurologists reviews and adjudicates all suspected cerebrovascular events. A diagnosis of CVD includes fatal or nonfatal CHD (recognized and unrecognized myocardial infarction, stable or unstable angina, and CHD death), cerebrovascular disease (stroke, transient ischemic attack, peripheral vascular disease [intermittent claudication], and heart failure. In our primary analyses, we focused on incidence of a first CVD event as defined above, consistent with numerous prior Framingham reports. In secondary analyses, we related plasma CETP activity to the incidence of a first “hard” CVD event, a composite end point that included fatal and nonfatal myocardial infarction, unstable angina, stroke, or congestive heart failure; this combination of events was chosen to correspond roughly to the primary outcome in the ILLUMINATE trial.8

Statistical Analyses

Given the near normal distribution of plasma CETP activity, we used untransformed values. We evaluated the correlation of plasma CETP activity with HDL-C using Pearson correlation coefficients. Because CETP activity distributions were quite similar in men and women, and we did not observe effect modification by sex on formal testing, all analyses were performed for pooled sexes to maximize statistical power. We modeled plasma CETP activity as a binary variable (dichotomized at the median) in primary analyses. A median cut point is reasonable from a physiological perspective because pharmacological inhibitors reduce plasma CETP activity by ~35% to 65% in a dose-dependent manner.26–29 and partial genetic CETP deficiency is associated with increased CVD risk17–21 typically associated with ~40% to 70% of normal activity.35–37 We also performed additional analyses modeling plasma CETP activity as a continuous variable.

We compared the cumulative incidence of CVD for groups with plasma CETP activity at or above versus below the median value (131 nmol/L per hour). After confirming that the assumption of proportionality of hazards was met, we used Cox proportional hazards regression to relate plasma CETP activity to incidence of a first CVD event on follow-up. Four sets of models were constructed hierarchically: (1) adjusted for age and sex; (2) additionally adjusted for baseline levels of standard CVD risk factors other than HDL-C (ie, systolic blood pressure, hypertension treatment, total cholesterol, smoking, and diabetes mellitus); (3) adjusted for baseline standard risk factors noted above and for HDL-C; and (4) adjusted for standard risk factors including HDL-C in time-dependent analyses with updating of risk factors every 4 years (including adjustments for the use of lipid-lowering treatment on follow-up). To gain insights into potential nonlinearity of associations between plasma CETP activity and incidence of CVD, we examined generalized additive Cox models using penalized splines. These analyses also facilitate assessment of the dose-response relation between plasma CETP activity and CVD incidence. We also performed sensi-
Recent reports have underscored the context-specific associations of altered CETP activity, depending on sex, the level of HDL-C, triglycerides, or absolute CVD risk. Therefore, we tested for effect modification by age, sex, high triglycerides (above the median was associated with a 25% to 28% lower risk of CVD consistently across the various models. In models incorporating both HDL-C and CETP activity, HDL-C was associated inversely with CVD incidence (hazard rate [HR] per mg/dL increment=0.98; 95% confidence interval [CI], 0.97 to 0.99; HR per SD=0.71; 95% CI, 0.61 to 0.82; P<0.0001).

When modeled as a continuous variable, a SD increment in plasma CETP activity was associated with a 12% to 14% lower risk of CVD. Regression splines confirmed the linearity of the inverse association of CETP activity and CVD incidence (Figure 2). In sensitivity analyses analyzing tertiles instead of a median cut point, the inverse relation of CETP activity and incident CVD was maintained (HR per tertile increment=0.77; 95% CI, 0.58 to 1.01). In other sensitivity analyses evaluating the entire sample with plasma activity analyses: evaluating tertiles of CETP activity (instead of the median cut point) and analyzing the entire sample with plasma CETP activity assay without the exclusion of outliers.

Table 1. Baseline Characteristics by Plasma CETP Activity Below vs At or Above the Median

<table>
<thead>
<tr>
<th>Plasma CETP Activity (Median=131 nmol/L per Hour)</th>
<th>Whole Sample</th>
<th>&lt;Median</th>
<th>≥Median</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>1978</td>
<td>989</td>
<td>989</td>
<td>0.02</td>
</tr>
<tr>
<td>Women, %</td>
<td>53.8</td>
<td>51.3</td>
<td>56.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Age, y</td>
<td>51±10</td>
<td>52±10</td>
<td>50±10</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±4.8</td>
<td>27.0±4.7</td>
<td>26.7±4.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±19</td>
<td>126±18</td>
<td>125±18</td>
<td>0.85</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79±10</td>
<td>79±10</td>
<td>79±10</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>15.6</td>
<td>16.3</td>
<td>15.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>23.3</td>
<td>24.7</td>
<td>21.9</td>
<td>0.73</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>4.1</td>
<td>3.9</td>
<td>4.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>205±38</td>
<td>203±37</td>
<td>207±39</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>50±15</td>
<td>50±15</td>
<td>50±14</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>131±35</td>
<td>129±34</td>
<td>134±35</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides, mg/dL, median (Q1–Q3)</td>
<td>98 (67–147)</td>
<td>103 (69–154)</td>
<td>93 (64–142)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lipid-lowering drugs, %</td>
<td>2.6</td>
<td>3.2</td>
<td>2.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma CETP activity, nmol/L per hour</td>
<td>149±85</td>
<td>80±28</td>
<td>218±62</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data are mean±SD unless indicated otherwise. Q1–Q3 indicates quartile 1 to quartile 3.

*P for comparison of CETP groups.

Results

The baseline characteristics of our sample overall and according to CETP activity are shown in Table 1. Participants with plasma CETP activity below the median were slightly older, were more likely to be men, and had slightly lower mean LDL-C but higher mean triglyceride levels compared with those with activity at or above the median. Although the 2 groups had similar HDL-C, overall CETP activity was related inversely to HDL-C in the sample (r=-0.07, P=0.001); this modest correlation is consistent with that observed in relatively normolipemic individuals.

On follow-up (mean, 15.1 years; maximum, 19.6 years), 320 participants (124 women) experienced a first CVD event. Table 2 and Figure 1 suggest that individuals with plasma CETP activity below the median experienced higher CVD incidence relative to those with activity at or above the median.

The results of analyses relating plasma CETP activity to CVD incidence are shown in Table 3. Plasma CETP activity at or above the median was associated with a 25% to 28% lower risk of CVD.
Without excluding outlier values, the results of our primary analyses remained robust.

Figure 3 displays the association of plasma CETP activity with incidence of hard CVD events (complementing analyses of all CVD events shown in Table 3). Plasma CETP activity at or above the median was associated with a 27% to 30% lower risk of CVD, consistent with the primary analyses. We did not observe any effect modification by age, sex, high triglycerides, low or high HDL-C, high LDL-C or apoB levels, or a high Framingham Risk Score (P values for all interactions >0.05).

Discussion

The discovery of CETP and the elucidation of its diverse roles in lipid physiology have fueled a contentious debate on its proatherosclerotic and antiatherosclerotic roles.

Genetic epidemiological studies also have not resolved the issue. Genetic investigations focusing on partial CETP deficiency states have yielded conflicting results, sometimes from the same study cohort. Experimental approaches are also challenged by the lack of CETP in some species, diverse cholesterol transport mechanisms, and a different time frame for development of atherosclerosis in susceptible animals compared with humans.

The controversy surrounding CETP inhibition as a strategy for raising HDL-C and lowering CVD risk has intensified further in light of recent disappointing clinical trial data associated with 1 of several newly synthesized inhibitors of CETP. Given this background, we evaluated prospectively the relations of plasma CETP activity to CVD incidence in a community setting.

Principal Findings

Our principal finding was an inverse relation of plasma CETP activity and CVD incidence. Although our findings are observational, the possibility that the observed relationship is causal one is strengthened by its biological plausibility.

Table 3. Association of Plasma CETP Activity With Incidence of CVD: Results of Multivariable Cox Regression

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma CETP activity as a binary variable, HR for CETP activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥median (&lt;median as referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>0.75 (0.60–0.94)</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable, adjusted for baseline risk factors* other than HDL-C</td>
<td>0.73 (0.59–0.92)</td>
<td>0.007</td>
</tr>
<tr>
<td>Multivariable, adjusted for baseline risk factors including HDL-C</td>
<td>0.72 (0.57–0.90)</td>
<td>0.004</td>
</tr>
<tr>
<td>Multivariable-adjusted time-dependent covariates†</td>
<td>0.73 (0.58–0.91)</td>
<td>0.006</td>
</tr>
<tr>
<td>Plasma CETP activity as a continuous variable, HR per SD increment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>0.88 (0.78–0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>Multivariable, adjusted for baseline risk factors* other than HDL-C</td>
<td>0.87 (0.78–0.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>Multivariable, adjusted for baseline risk factors including HDL-C</td>
<td>0.86 (0.76–0.97)</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable-adjusted time-dependent covariates</td>
<td>0.88 (0.78–0.98)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Age, sex, total cholesterol, smoking, diabetes mellitus, systolic blood pressure, use of antihypertensive medications.

†All covariates including HDL-C updated every 4 years at Framingham Heart Study examinations (also adjusted for lipid-lowering drug use on follow-up).
below), the demonstration of a linear dose response (regression splines), the presence of a temporal relationship (plasma CETP activity was assessed before CVD events), and the consistency of the association in multiple analyses (incorporating covariates sequentially, including time-dependent models; modeling plasma CETP activity as a binary and as a continuous variable; and evaluation of all CVD and hard CVD events). The strength of the association (a 25% to 28% lower risk with plasma CETP activity values above the median [which corresponds to a hazard of 1.35 to 1.40 for plasma CETP activity values below the median, relative to those above]) is of a magnitude similar to that reported for partial CETP deficiency in some reports.\textsuperscript{17,18,20} We did not find evidence of effect modification by age, sex, HDL-C, LDL-C, or triglyceride levels and the global CVD risk (as estimated by the Framingham Risk Score), in contrast to other reports that noted inverse associations of CETP levels (or of related genotypes) and CVD risk in select subgroups.\textsuperscript{16,17,19,23}

Data on the relations of CETP mass and CVD are conflicting,\textsuperscript{22–24} as noted earlier, in part related to modest numbers of CVD events and varying study designs. We used a plasma CETP activity assay that was already available in our study cohort at an earlier examination\textsuperscript{27} (as opposed to CETP mass assays used by several investigators\textsuperscript{22–24}), which facilitated a long-term prospective time-to-event analysis (20 years of follow-up) in this large community-based sample. It is noteworthy that circulating CETP mass and activity have been reported to be highly correlated (\(r = 0.85\)) in several reports.\textsuperscript{51–53}

We observed a very modest inverse relation of plasma CETP activity with HDL-C in our sample. This may be in part because HDL-C concentrations are determined by several enzymatic processes in addition to CETP (such as lecithin:cholesterol acyltransferase, hepatic lipase, and phospholipid transfer protein),\textsuperscript{54,55} or it may arise because we measured CETP activity using a fabricated substrate that does not interact with the natural substrate in this reaction (ie, plasma HDL-CE). Of note, the relations of CETP activity and HDL-C have been inconsistent in the literature, with some investigations reporting no correlation,\textsuperscript{54,56} whereas others have noted modest inverse correlations of a magnitude consistent with our findings.\textsuperscript{38}

The failure of the CETP inhibitor torcetrapib has drawn attention to the complex and potential beneficial effects of CETP on reverse cholesterol transport at several levels. Irrespective of the toxicity issues of torcetrapib, in clinical trials this agent produced a substantial increase in HDL-C (as predominantly CEs) but did not appear to reduce atherosclerosis in either carotid\textsuperscript{50} or coronary vessels.\textsuperscript{14} Studies by Schwartz et al\textsuperscript{57} have shown with multicompartmental analysis in humans that very little CE is transported directly from HDL to the liver but instead is predominantly delivered to the liver from apoB-containing lipoproteins. This is in contrast to the fate of HDL-unesterified cholesterol, which is readily taken up by the liver and secreted in bile or utilized as the primary precursor for bile acid synthesis.\textsuperscript{58} In summary, these kinds of results would support raising levels of HDL-C but not blocking a natural egress pathway of HDL-CE to apoB-containing lipoprotein for delivery to the liver. Given the development of agents in the same class that seem to lack the off-target effects of torcetrapib, it is important to conduct additional studies to confirm our findings.

**Strengths and Limitations**

The moderate-sized community-based sample of middle-aged men and women, prospective design, and assessment of CVD outcomes blinded to plasma CETP activity levels strengthen our study. Several limitations must be acknowledged. First, assessment of plasma CETP activity in vitro is challenging.\textsuperscript{59} We used a standardized assay, and any measurement error is likely random, biasing us toward the null hypothesis of no association of CETP activity with CVD incidence. The ex vivo assessment of CETP activity may not reflect in vivo activity. Furthermore, the activity assay used estimated rates of mass transfer of CEs from donor particles (phospholipids) to acceptor particles (VLDL) and therefore reflects only 1 aspect of CETP activity. CETP is involved in lipid transfer reactions involving several different lipid particles.\textsuperscript{43,59,60} Second, we had single-occasion measurements of CETP activity, which would result in an underestimation of the strength of the association (regression dilution bias).\textsuperscript{61} Third, given the observational study design, our results cannot be extrapolated directly to the potential effects of
pharmacological inhibition of CETP activity with the use of available agents. Finally, our sample was middle-aged, with an intermediate pretest probability of CVD, and exclusively white, which would limit the generalizability of our results to age groups or ethnicities not studied.

Conclusions

In our prospective investigation of a moderate-sized community-based sample, lower plasma CETP activity was associated with greater risk of CVD. These observations, if confirmed, call into question the strategy of pharmacological inhibition of CETP to lower CVD risk.

Sources of Funding

This work was supported by the National Heart, Lung, and Blood Institute’s Framingham Heart Study (contract No. N01-HC-25195), HL54776, 2 K24 HL04334 (Dr Vasan), and contract 53-K06-5-10 from the US Department of Agriculture Research Service.

Disclosures

None.

References

14. Vasan et al. CETP Activity and CVD Incidence
Inhibiting cholesteryl ester transfer protein (CETP) activity raises high-density lipoprotein cholesterol levels and may be cardioprotective. However, an initial clinical trial with a CETP inhibitor was stopped prematurely because of increased cardiovascular disease (CVD) events in treated patients. Data relating circulating CETP mass to CVD incidence in the community are also conflicting. Therefore, we related routinely assayed plasma CETP activity to the incidence of CVD events on follow-up (average, 15 years) in the offspring cohort of the Framingham Heart Study. In multivariable analyses adjusted for standard CVD risk factors, plasma CETP activity was related inversely to the incidence of CVD events, a finding that remained robust in time-dependent models updating CVD risk factors every 4 years on follow-up and was maintained in analyses of incident “hard” CVD events. These observational data based on prospective follow-up of a large community-based sample require confirmation. If confirmed, our findings would call into question the use of CETP inhibition as a strategy for lowering CVD risk.

**CLINICAL PERSPECTIVE**


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Appendix 1. Comparison of participants with and without plasma CETP activity measurements at the Fourth Offspring examination.

<table>
<thead>
<tr>
<th></th>
<th>Study sample, with plasma CETP activity (n=1978)</th>
<th>Plasma CETP activity not assayed (n=1623)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, %</td>
<td>53.8</td>
<td>52.8</td>
</tr>
<tr>
<td>Age, years</td>
<td>51±10</td>
<td>51±10</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±4.8</td>
<td>26.7±4.9</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±19</td>
<td>127±19</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79±10</td>
<td>80±10</td>
</tr>
<tr>
<td>Hypertension treatment, (%)</td>
<td>32.9</td>
<td>34.5</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>205±38</td>
<td>206±39</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>23.3</td>
<td>25.3</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>4.1</td>
<td>5.5*</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>50±15</td>
<td>50±15</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>131±35</td>
<td>132±36</td>
</tr>
</tbody>
</table>

*p=0.04 for differences between groups. For the remaining variables, there were no statistically significant differences between the two groups (all p-values exceeded 0.05).
Appendix 2. Plasma CETP activity assay.

Briefly, for each sample assayed, 10 µL of plasma was diluted (1:10) in 90 µL of sample buffer (10 mmol/L Tris, 150 mmol/L NaCl, and 2 mmol/L EDTA, pH 7.4). In a fluorescence compatible microtiter plate (Dynex Laboratories), 20 µL of the plasma dilution was combined with 4 µL of donor and 4 µL of acceptor in a total volume of 200 µL and incubated for 3 hours at 37°C. The assay was read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm. A standard curve was used, according to the manufacturer’s guidelines, to derive the relation between fluorescence intensity and cholesteryl ester mass transfer. Plasma controls were run in each plate to account for plate-to-plate variation. For standardization, the unquenched fluorescence intensity of the fluorescent cholesteryl ester contained within the donor particle core was determined by dispersing 5 µL of donor (fluorescent cholesteryl ester concentration 146 mg/mL, as reported by the manufacturer) in 2 mL of 100% isopropanol. Serial dilutions of the dispersion were made to generate a standard curve of fluorescence intensity (excitation 465 nm/emission 535 nm) versus mass of fluorescent cholesteryl ester. The fluorescence intensity transferred in the assay of plasma samples was applied to the standard curve to determine cholesteryl ester mass transfer.