Plasma Levels of Cholesteryl Ester Transfer Protein and the Risk of Future Coronary Artery Disease in Apparently Healthy Men and Women

The Prospective EPIC (European Prospective Investigation into Cancer and nutrition)—Norfolk Population Study

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Background—Low plasma levels of cholesteryl ester transfer protein (CETP) are associated with elevated levels of HDL cholesterol (HDL-C), but it remains unclear whether this translates into a concomitant reduction in the risk of coronary artery disease (CAD). Evidence exists that the effect of CETP depends on metabolic context, in particular on triglyceride levels.

Methods and Results—A nested case-control study was performed in the prospective EPIC-Norfolk cohort study. Cases were apparently healthy men and women aged 45 to 79 years who developed fatal or nonfatal CAD during follow-up. Control subjects were matched by age, sex, and enrollment time. CETP levels were not significantly different between cases and controls (4.0±2.2 versus 3.8±2.1 mg/L, P=0.07). CETP levels were significantly related to plasma levels of total cholesterol, LDL cholesterol, and HDL-C. The risk of CAD increased with increasing CETP quintiles (P for linearity=0.02), such that subjects in the highest quintile had an adjusted OR of 1.43 (95% CI 1.03 to 1.99, P=0.03) versus those in the lowest. Among individuals with triglyceride levels below the median (1.7 mmol/L), no relationship between CETP levels and CAD risk was observed (P for linearity=0.5), but this relationship was strong among those with high triglyceride levels (P for linearity=0.02), such that those in the highest CETP quintile had an OR of 1.87 (95% CI 1.06 to 3.30, P=0.02).

Conclusions—Elevated CETP levels are associated with an increasing risk of future CAD in apparently healthy individuals, but only in those with high triglyceride levels. (Circulation. 2004;110:1418-1423.)

Key Words: cholesterol • lipoproteins • coronary disease • atherosclerosis

Among numerous genetic and lifestyle parameters, dyslipidemia is a prominent risk factor for coronary artery disease (CAD). In the past decade, lowering of LDL cholesterol (LDL-C) has been established as the principal target for therapeutic intervention in dyslipidemia and now constitutes the foundation of CAD prevention.1-3 However, because statin therapy typically yields risk reduction of ≈30%, there is great potential for additional risk reduction through modulation of cardiovascular risk factors other than LDL-C. In particular, prospective epidemiological studies have consistently shown that a decreased concentration of HDL cholesterol (HDL-C) is a strong and independent risk factor for the development of CAD.4,5 As a consequence, pharmacological intervention to raise HDL-C currently enjoys significant interest.6

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Cholesteryl ester transfer protein (CETP) plays a central role in HDL-C metabolism by shuttling cholesteryl esters (CEs) from HDL particles to apolipoprotein B (apoB)–containing particles in exchange for triglycerides.7 Plasma levels of CETP have an inverse relationship with HDL-C levels,8 and in Japanese populations, genetic CETP deficiency has been identified as an important cause of high
HDL-C levels. These observations have prompted the realization of pharmacological CETP inhibitors, which are effective in raising HDL-C levels. However, it remains uncertain whether the increased HDL-C levels induced by pharmacological inhibition of CETP activity translate into a CAD risk reduction.

Evidence exists that the consequences of CETP activity may depend on the metabolic setting, particularly on triglyceride levels, which are precursors of small dense LDL particles, the most atherogenic LDL subclass. CETP further contributes to the formation of small dense LDL by preferential CE transfer from HDL to small dense LDL species and enhanced transfer to apoB-containing VLDL particles. Thus, the potential detrimental effect of high CETP levels in terms of CAD risk may depend on triglyceride levels.

It is remarkable that despite the fact that pharmacological CETP inhibition is already being assessed in human trials, no epidemiological evidence exists to support a direct relationship. CETP concentrations were measured with a validated 2-antibody sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and biotinylated monoclonal antibodies against CRP (Sanquin Research) were used as the detecting antibody. Results were related to a standard that consisted of commercially available CRP (Behring-Werke AG). The lower detection limit was 0.1 mg/L. CETP concentrations were measured with a validated 2-antibody sandwich-type ELISA. Samples were measured as duplicates, and the assay was repeated if the intra-assay variation was >10%. Mean of duplicates was used as a variable in subsequent analyses. Samples were analyzed in random order to avoid systemic bias.

Statistical Analysis
Baseline characteristics were compared between cases and controls with a mixed-effect model for continuous variables or conditional logistic regression for categorical variables. Because triglyceride levels had a skewed distribution, values were log-transformed before statistical analysis. Our primary objective was to evaluate the relationships between CETP plasma levels, cardiovascular risk factors, and the risk of CAD. Therefore, CETP levels were categorized into quintiles based on the distribution in the control subjects. Mean levels of traditional cardiovascular risk factors were calculated per CETP quintile. Conditional logistic regression analysis was used to calculate ORs and corresponding 95% CIs as an estimate of the relative risk of CAD. CETP concentrations were analyzed as categorical variables after division into quintiles, with the lowest quintile used as the reference category. ORs were calculated taking into account the matching for age and sex and were adjusted for the following cardiovascular risk factors: smoking, systolic blood pressure, diabetes, body mass index (BMI), CRP levels, and fasting time (the time between the last meal and the moment of drawing blood). ORs also were calculated after additional adjustment for LDL-C, HDL-C, and the ratio LDL-C/HDL-C. To study a possible interaction with triglyceride levels, participants were stratified according to the median triglyceride concentration. In these 2 strata, CETP levels were divided into quintiles according to the distribution in the control subjects. The mean LDL-C/HDL-C ratio and the OR for future CAD were calculated per quintile, with the lowest CETP quintile used as the reference. Subsequently, we compared the regression slopes for both LDL-C/HDL-C ratio and CAD risk between individuals above and below the median triglyceride level. Statistical analyses were performed with SPSS software (version 10.1). A probability value <0.05 was considered significant.

**Participants**
For the present nested case-control study, we identified 755 apparently healthy individuals who ultimately developed fatal or nonfatal CAD during follow-up. Apparently healthy individuals were defined as study participants who did not report a history of heart attack or stroke at the baseline clinic visit. Control subjects were apparently healthy study participants who remained free of CAD during follow-up. Two controls were matched to each case by sex, age (within 5 years), and date of visit (within 3 months).

**Biochemical Analyses**
Nonfasting blood samples were taken by venepuncture into plain and citrate bottles. Blood samples were processed for assay at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. Serum levels of total cholesterol, HDL-C, and triglycerides were measured in fresh plasma samples with the RA 1000 (Bayer Diagnostics), and LDL-C levels were calculated with the Friedewald formula. C-reactive protein (CRP) and CETP concentrations were measured on thawed plasma from cases and controls. CRP levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and biotinylated monoclonal antibodies against CRP (Sanquin Research) were used as the detecting antibody. Results were related to a standard that consisted of commercially available CRP (Behring-Werke AG). The lower detection limit was 0.1 mg/L. CETP concentrations were measured with a validated 2-antibody sandwich-type ELISA. Samples were measured as duplicates, and the assay was repeated if the intra-assay variation was >10%. Mean of duplicates was used as a variable in subsequent analyses. Samples were analyzed in random order to avoid systemic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

**Methods**

**Study Design**
We performed a nested case-control study among participants of the EPIC (European Prospective Investigation into Cancer and Nutrition)–Norfolk cohort study, a prospective population study of 25,663 men and women aged between 45 and 79 years, resident in Norfolk, United Kingdom, who completed a baseline questionnaire survey and attended a clinic visit. EPIC-Norfolk is part of the 9-country collaborative EPIC study designed to investigate dietary and other determinants of cancer. Additional data were obtained to enable the assessment of determinants of other diseases. The study cohort was closely similar to UK population samples with regard to many characteristics, including anthropometry, blood pressure, and lipids, but with a lower proportion of smokers.

Participants were recruited by post from age-sex registers of general practices. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire, and additional data collection was performed by trained nurses at a clinic visit as described previously. All individuals have been flagged for mortality at the UK Office of National Statistics, with vital status ascertained for the entire cohort. Death certificates for all decedents were coded by trained necrologists according to the International Classification of Diseases (ICD) 9th revision. Death was considered due to CAD if the underlying cause was coded as ICD 410 to 414. In addition, participants admitted to a hospital were identified by their unique National Health Service number by data linkage with ENCORE (East Norfolk Health Authority database), which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as an underlying cause. We report results with follow-up up to January 2003, an average of ~6 years. The Norwich District Health Authority Ethics Committee approved the study, and all participants gave signed informed consent.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=1400)</th>
<th>Cases (n=735)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.9±7.6</td>
<td>64.9±7.7</td>
<td>Matched</td>
</tr>
<tr>
<td>Male sex</td>
<td>927 (66.2)</td>
<td>486 (66.2)</td>
<td>Matched</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
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<tr>
<td>Never</td>
<td>560 (40.4)</td>
<td>222 (30.5)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>696 (50.3)</td>
<td>393 (53.8)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>129 (9.3)</td>
<td>113 (15.4)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4±3.5</td>
<td>27.3±3.8</td>
<td>&lt;0.0001</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.27±1.15</td>
<td>6.49±1.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.08±1.01</td>
<td>4.23±1.03</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.36±0.40</td>
<td>1.26±0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7 (1.2–2.4)</td>
<td>1.9 (1.4–2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140±19</td>
<td>145±19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84±11</td>
<td>86±12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35 (2.5)</td>
<td>53 (7.2)</td>
<td></td>
</tr>
<tr>
<td>CRP, pg/mL</td>
<td>3.0±5.0</td>
<td>4.3±5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CETP, mg/L</td>
<td>3.8±2.1</td>
<td>4.0±2.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, n (%), or median (interquartile range). Means, percentages, and medians may be based on fewer observations than the indicated number of subjects.

Results

Plasma was available for 735 cases and 1400 matched controls; 665 cases were matched to 2 controls each, and 70 cases could be matched to 1 control each. The cases and controls for whom no plasma was available did not differ in any aspect from those for whom plasma was available (data not shown). Matching ensured that age and sex were comparable between cases and controls. As expected, individuals who developed CAD during follow-up were more likely than controls to smoke and to have diabetes (Table 1). Levels of total cholesterol, LDL-C, triglycerides, systolic and diastolic blood pressure, BMI, and CRP were significantly higher in cases than controls, whereas HDL-C levels were significantly lower.

For the CETP assay, the mean intra-assay variation between duplicates was 7.8%. CETP levels were higher in cases than controls (4.0±2.2 versus 3.8±2.1 mg/L), but this did not reach statistical significance (P=0.07). CETP plasma levels were significantly correlated with total cholesterol, LDL-C, HDL-C, and triglyceride levels but not with BMI (Table 2). With increasing CETP quintiles, the OR for future CAD, adjusted for smoking, systolic blood pressure, diabetes, BMI, CRP, and fasting time, increased in a linear pattern (P for linearity=0.02; Table 3). Individuals in the highest CETP quintile had a 1.5-fold increased risk of future CAD compared with those in the lowest quintile (OR 1.43, 95% CI 1.03 to 1.99, P=0.03).

**Effect Modification by Triglyceride Levels**

Among individuals with triglyceride levels below the median (1.7 mmol/L), CETP plasma levels did not differ between cases and controls (3.9±2.3 versus 3.9±2.3 mg/L, P=0.5). However, among those with triglyceride levels above the median, CETP levels were significantly higher in cases than in controls (4.1±2.3 versus 3.8±2.0 mg/L, P=0.036). Subsequently, we evaluated whether a statistical interaction existed between triglyceride levels (below or above the median) and CETP levels (as a continuous variable) for the ratio LDL-C/HDL-C, which was the strongest correlate of CETP levels (Table 2). We calculated the regression slopes of CETP levels on the ratio LDL-C/HDL-C for individuals above and below the median triglyceride level and found that these regression slopes differed significantly (P=0.04).

We then investigated whether a similar interaction existed for future CAD risk. Among individuals with triglyceride levels below the median, the risk of future CAD did not increase in increasing CETP quintiles (P for linearity=0.5, Figure). In contrast, CAD risk did increase among individuals with high triglyceride levels (P for linearity=0.02). Compared with individuals in the lowest quintile, those in the second, third, fourth, and fifth quintiles had the following respective ORs for future CAD: 1.50 (95% CI 0.84 to 2.67), 1.51 (95% CI 0.84 to 2.69), 1.84 (95% CI 1.02 to 3.32), and 1.87 (95% CI 1.06 to 3.30; Figure). The interaction term between triglyceride levels (above or below the median) and CETP levels did not reach statistical significance when CETP levels were entered as quintiles (P=0.08), but it did reach

<table>
<thead>
<tr>
<th>CETP Quintile, mg/L</th>
<th>&lt; 2.4</th>
<th>2.4–2.9</th>
<th>3.0–3.7</th>
<th>3.8–4.9</th>
<th>&gt;4.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.0±1.1</td>
<td>6.3±1.1</td>
<td>6.3±1.2</td>
<td>6.6±1.3</td>
<td>6.6±1.2</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.8±1.0</td>
<td>4.0±0.9</td>
<td>4.1±1.0</td>
<td>4.3±1.0</td>
<td>4.4±1.0</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.38±0.42</td>
<td>1.33±0.34</td>
<td>1.32±0.38</td>
<td>1.30±0.40</td>
<td>1.29±0.40</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.0±1.2</td>
<td>2.0±1.1</td>
<td>2.0±1.0</td>
<td>2.1±1.1</td>
<td>2.1±1.2</td>
</tr>
<tr>
<td>Ratio LDL-C/HDL-C</td>
<td>2.9±1.1</td>
<td>3.2±1.1</td>
<td>3.3±1.2</td>
<td>3.6±1.3</td>
<td>3.7±1.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9±3.6</td>
<td>26.5±3.5</td>
<td>26.7±3.9</td>
<td>26.8±3.6</td>
<td>26.8±3.5</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD per quintile. *P value for linearity between CETP quintiles and risk factor levels. †P value for Spearman’s correlation between CETP quintiles and risk factor levels.
borderline statistical significance when CETP levels were entered as a continuous variable ($P=0.047$).

On adjustment for LDL-C levels, the relationship between CETP levels and CAD risk among people with high triglyceride levels remained statistically significant (OR 1.86, 95% CI 1.02 to 3.68, $P=0.045$ for people in the highest CETP quintile compared with those in the lowest one). However, on additional adjustment for HDL-C levels and on additional adjustment for the ratio LDL-C/HDL-C, the relationship between CETP levels and CAD risk was attenuated and became nonsignificant; OR 1.64 (95% CI 0.86 to 3.13, $P=0.1$) and OR 1.60 (95% CI 0.82 to 3.11, $P=0.2$), respectively for those in the highest CETP quintile compared with those in the lowest one. These results suggest that a potential effect of CETP levels on CAD risk may be mediated via its effect on HDL-C levels or on the ratio LDL-C/HDL-C.

### Discussion

In a large prospective study among apparently healthy men and women, we observed that CETP plasma levels have a positive linear correlation with LDL-C plasma levels and, in contrast, a negative linear correlation with HDL-C plasma levels. After adjustment for traditional nonlipid risk factors, the risk of future CAD increased with increasing CETP quintiles. The relationship between elevated CETP levels and increased risk of CAD was linear and was confined to individuals with elevated triglyceride levels, ie, above the median 1.7 mmol/L. Finally, we observed that the elevated CAD risk associated with higher CETP levels was attenuated after additional adjustment for HDL-C or for the ratio LDL-C/HDL-C.

### CETP Plasma Levels and HDL-C Levels

In this large group of apparently healthy individuals, we observed a significant negative correlation between CETP levels and HDL-C levels. The literature on this issue is not consistent\(^8,28-34\); however, most of these studies were performed in small groups of individuals who were selected on the basis of abnormal lipid levels, lipid disorders, diabetes, or CAD. In contrast, the present results are consistent with our recent meta-analysis, which showed that throughout a large number of studies in both healthy and diseased populations, CETP gene variants associated with decreased CETP activity also were associated with elevated HDL-C levels\(^35\). This observed correlation is also consistent with the fact that individuals with genetic CETP deficiency invariably present with high HDL-C levels\(^36-38\) and with the fact that pharmacological CETP inhibition raises HDL-C levels\(^10-12\).

### CETP Plasma Levels and Risk of CAD

The present study shows that increasing CETP levels are associated with an increased risk of future CAD in apparently healthy individuals. To the best of our knowledge, only one small cross-sectional study, in Chinese individuals, has also investigated the relationship between CETP plasma levels and cardiovascular end points. In line with our observations, those investigators reported that CETP plasma levels were higher in a group of patients who had myocardial infarction than in healthy controls\(^34\). Two other, more recent reports studied the relationship between CETP concentration and...
surrogate markers for atherosclerosis. Using consecutive coronary angiography, we have shown that among men with established CAD, those with high CETP levels have increased progression of coronary atherosclerosis compared with those with low CETP levels.\textsuperscript{29} We have also used B-mode ultrasound to measure the intima-media thickness of the carotid artery in patients with familial hypercholesterolemia and have shown again that elevated CETP levels were associated with increased progression of atherosclerosis.\textsuperscript{28} In addition to studies among healthy individuals and patients with CAD, studies in subjects with genetic CETP deficiency also have provided insight into the relation between CETP and atherosclerosis. In this regard, the CETP (+1)G$>$A and D442G gene variants have been the subject of extensive studies. A large study among Japanese-American men in Honolulu indicated that carriers of these defects had a lower risk of CAD, but this was not statistically significant.\textsuperscript{30}

**Risk Modification by Triglyceride Levels**

We observed that the relationship between CETP levels and CAD risk was strong among individuals with high triglyceride levels but absent among those with low triglyceride levels. This analysis was predefined and was driven by the evidence that CETP-facilitated transfer of CEs to apoB-containing particles is mediated by increased triglyceride levels in both humans\textsuperscript{13–15} and mice.\textsuperscript{16,17} As triglyceride levels rise, the principal lipoprotein subfraction to accumulate is VLDL\textsubscript{1} particles. These particles are very triglyceride rich and can therefore act as potent acceptors of CETP-facilitated transfer of CE from both HDL and LDL particles.\textsuperscript{20} In exchange, CETP redistributes the high triglyceride load in these VLDL\textsubscript{1} particles to both HDL and LDL particles. As a result, LDL particles become triglyceride enriched, which makes them good targets for hepatic lipase activity, which, in turn, can lead to the formation of small dense LDL.\textsuperscript{18,19}

**Considerations**

Certain aspects of the present study merit further consideration. First, plasma levels of CETP were determined in a nonfasting sample, which would influence triglyceride levels and CETP concentrations\textsuperscript{40}; however, this would introduce an increased random measurement error, which is likely to lead to an underestimation of any relationship and therefore does not negate our findings. Second, the present data do not allow us to study the causality of the relationship between CETP and CAD. We cannot exclude the possibility that in this population study, CETP concentration was a marker of HDL-C levels rather than an effector, although this hypothesis would not be consistent with observations that pharmacological inhibition of CETP results in higher HDL-C levels.\textsuperscript{10–12} Finally, the results of the present analysis raise several issues about the potential effects of currently tested CETP inhibitors. First, we have studied apparently healthy individuals, although CETP inhibitors will be used (most likely in combination with statins) in dyslipidemic people and people at high cardiovascular risk. Second, we have studied CETP under physiological conditions in which CETP activity and CETP concentration are assumed to be strongly correlated.\textsuperscript{32} This contrasts with the reduced CETP activity on pharmacological inhibition that is accompanied by an increase in CETP plasma concentration, probably because the interaction of the small molecules with the CETP protein disturbs CETP clearance from the circulation.\textsuperscript{10,11} When these considerations are taken into account, care is warranted when extrapolating our finding that low CETP concentrations are associated with a moderately reduced risk for CAD in healthy individuals to the putative effects of CETP inhibition on CAD risk in patients. The ongoing trials assessing the effect of CETP inhibitors on surrogate markers of CAD and clinical end points will have to answer this question.

**Conclusions**

We conclude that increasing concentrations of CETP are associated with an increasing risk of future CAD in apparently healthy individuals. The observed relationship was linear and was confined to individuals with elevated triglyceride levels (above the median 1.7 mmol/L). Among apparently healthy individuals with elevated triglyceride levels, those in the highest CETP quintile had an OR of 1.87 (95% CI 1.06 to 3.30, \(P=0.02\)) compared with those in the lowest quintile. These prospective data support the hypothesis that pharmacological CETP inhibition may reduce the risk of CAD in humans, but only in those with high triglyceride levels.

**Acknowledgments**

We thank the participants, general practitioners, and staff in EPIC-Norfolk. EPIC-Norfolk is supported by program grants from the Medical Research Council UK and Cancer Research UK and with additional support from the European Union, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and the Wellcome Trust. Dr Kastelein is an established investigator (2000 D039) and Dr Jukema is an established clinical investigator (2001 D032) of the Netherlands Heart Foundation, The Hague, the Netherlands.

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