Genetic and biochemical risks factors in coronary artery disease

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Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women; the prospective EPIC-Norfolk population study
Abstract

Background

Low plasma levels of cholesteryl ester transfer protein (CETP) are associated with elevated levels of high-density lipoprotein cholesterol (HDL-c), but it remains unclear whether this translated 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 45-79 who developed fatal or non-fatal CAD during follow-up. Controls were matched by age, sex and enrolment 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 with plasma levels of total cholesterol, LDL-c, and HDL-c. The risk of CAD increased with increasing CETP quintiles (p linearity = 0.02), such that subjects in the highest quintile had an adjusted odds ratio of 1.43 (1.03-1.99, 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 linearity = 0.5), but this relationship was strong among those with high triglyceride levels (p linearity = 0.02), such that those in the highest CETP quintile had an OR of 1.87 (95%CI = 1.06-3.30, 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.
Introduction
Among numerous genetic and lifestyle parameters, dyslipidaemia is the prominent risk factor for coronary artery disease (CAD). In the past decade, lowering of low-density lipoprotein cholesterol (LDL-c) has been established as the principal target for therapeutic intervention in dyslipidaemia, and now constitutes the foundation of CAD prevention. However, since statin therapy typically yields risk reduction of approximately 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 high-density lipoprotein cholesterol (HDL-c) is a strong and independent risk factor for the development of CAD. As a consequence, pharmacological intervention to raise HDL-c currently enjoys significant interest.

Cholesterol ester transfer protein (CETP) plays a central role in HDL-c metabolism by shuttling cholesteryl esters (CE) from HDL particles to apolipoprotein B (apoB)-containing particles in exchange for triglycerides. Plasma levels of CETP have an inverse relationship with HDL-c levels, 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. Transfer of cholesteryl ester (CE) out of HDL particles is determined not only by the CETP concentration, but even more by the concentration of the acceptors of the transfer reaction, i.e. triglyceride-rich lipoproteins such as very low-density lipoprotein (VLDL). As triglyceride levels rise, VLDL particles are the principal lipoprotein subfraction to accumulate, shifting the preferential net CE flux from LDL particles to VLDL. This shift favours the formation of CE-depleted and triglyceride-enriched LDL particles which are precursors of small dense LDL particles, the most atherogenic LDL subfraction. 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. 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 between CETP levels and CAD risk. It was therefore our objective to study the prospective relationship between plasma levels of CETP and the risk of future CAD. In addition, we assessed whether this relationship depends on triglyceride levels.
Methods

Study design

We performed a nested case-control study among participants of the EPIC-Norfolk cohort study (EPIC, European Prospective Investigation into Cancer and Nutrition), a prospective population study of 25,663 men and women aged between 45 and 79 years, resident in Norfolk, UK, who completed a baseline questionnaire survey and attended a clinic visit. EPIC-Norfolk is part of the nine-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 respect 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 previously described. All individuals have been flagged for mortality at the UK Office of National Statistics, with vital status ascertained for the

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Controls</th>
<th>Cases</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1400</td>
<td>735</td>
<td></td>
</tr>
<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 - 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>&lt; 0.0001</td>
</tr>
<tr>
<td>- Current</td>
<td>129 (9.3)</td>
<td>113 (15.4)</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4 ± 3.5</td>
<td>27.3 ± 3.8</td>
<td>&lt; 0.0001</td>
</tr>
<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 cholesterol, mmol/l</td>
<td>4.08 ± 1.01</td>
<td>4.23 ± 1.03</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL cholesterol, 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, mmHg</td>
<td>140 ± 19</td>
<td>145 ± 19</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</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>&lt; 0.0001</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). LDL indicates low-density lipoprotein; HDL indicates high-density lipoprotein; CETP indicates cholesteryl ester transfer protein. Means, percentages and medians may be based on fewer observations than the indicated number of subjects.
entire cohort. Death certificates for all decedents were coded by trained
nosologists according to the International Classification of Diseases (ICD)
9th revision. Death was considered due to CAD if the underlying cause
was coded as ICD 410-414. In addition, participants admitted to hospital
were identified using 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 underlying cause.
We report results with follow-up up to January 2003, an average of about
6 years. The study was approved by the Norwich District Health Authority
Ethics Committee and all participants gave signed informed consent.

Participants
For the present nested case-control study, we identified 755 apparently
healthy individuals but who did develop fatal or non-fatal 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. Controls 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
Non-fasting 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, Basingstoke, UK),
and LDL-c levels were calculated with the Friedewald formula. C-reactive
protein (CRP) and CETP concentrations were measured on thawed frozen
plasma from cases and controls. C-reactive protein levels were measured
with a sandwich-type ELISA in which polyclonal rabbit anti-C-reactive
protein antibodies were used as catching antibodies and biotinylated
monoclonal antibodies against C-reactive protein (Sanquin Research,
Amsterdam, the Netherlands) as the detecting antibody. Results were
related to a standard consisting of commercially available C-reactive protein
(Behringwerke AG, Marburg, Germany). The lower detection limit was 0.1
mg/l. CETP concentrations were measured with a validated two-antibody
sandwich-type ELISA. Samples were measured as duplicates and the assay
was repeated if the intra-assay variation was >10%. The mean of duplicates
was used as 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.
Table 2. Distribution of cardiovascular risk factors by CETP quintiles

<table>
<thead>
<tr>
<th>CETP quintile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P*</th>
<th>R</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, mg/l</td>
<td>&lt; 2.4</td>
<td>2.4 - 2.9</td>
<td>3.0 - 3.7</td>
<td>3.8 - 4.9</td>
<td>&gt; 4.9</td>
<td></td>
<td>0.193</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Subjects</td>
<td>424</td>
<td>385</td>
<td>433</td>
<td>445</td>
<td>448</td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
<td>&lt; 0.0001</td>
<td>0.193</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, 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>
<td>&lt; 0.0001</td>
<td>0.218</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, 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>
<td>&lt; 0.0001</td>
<td>-0.091</td>
<td>&lt; 0.0001</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>
<td>0.06</td>
<td>0.041</td>
<td>0.06</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>
<td>&lt; 0.0001</td>
<td>0.231</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Body mass index, 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>
<td>0.9</td>
<td>0.000</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD per quintile. P* indicates p-value for linearity between CETP quintiles and risk factor levels; P† indicates p-value for Spearman's correlation between CETP quintiles and risk factor levels; R indicates Spearman's correlation between CETP quintiles and risk factor levels.
Statistical analysis
Baseline characteristics were compared between cases and controls using a mixed effect model for continuous variables\(^{25}\) or conditional logistic regression for categorical variables.\(^{26,27}\) 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 controls. Mean levels of traditional cardiovascular risk factors were calculated per CETP quintile. Conditional logistic regression analysis was used to calculate odds ratios (OR) and corresponding 95% confidence intervals (95%CI) as an estimate of the relative risk of CAD. CETP concentrations were analysed as categorical variables after division into quintiles, using the lowest quintile as reference category. ORs were calculated taking into account the matching for age and sex, and were adjusted for the following cardiovascular risk factors: smoking (never, past, current), 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 were also calculated after additional adjustment for LDL-c, HDL-c, and the ratio LDL-c / HDL-c. In order to study a possible interaction with triglyceride levels, participants were stratified according to the median triglyceride concentration. In these two strata, CETP levels were divided into quintiles according to the distribution in the controls. The mean LDL-c / HDL-c ratio and the OR for future CAD were calculated per quintile, using the lowest CETP quintile as 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 using SPSS software (version 10.1, Chicago, Illinois). A p-value less than 0.05 was considered significant.

Results
Plasma was available for 735 cases and 1400 matched controls; 665 cases were matched to two controls and 70 cases could be matched to 1 control. 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 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 mg/l 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
Tabl ee  3 . Odds  ratios  fo r  futur e  CA D  accordin g  t o  CET P  quintil e

<table>
<thead>
<tr>
<th>CETP quintile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, mg/l</td>
<td>&lt; 2.4</td>
<td>2.4 - 2.9</td>
<td>3.0 - 3.7</td>
<td>3.8 - 4.9</td>
<td>&gt; 4.9</td>
<td></td>
</tr>
<tr>
<td>Cases / controls</td>
<td>144 / 280</td>
<td>120 / 265</td>
<td>143 / 290</td>
<td>156 / 289</td>
<td>172 / 276</td>
<td></td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.00</td>
<td>0.99</td>
<td>1.04</td>
<td>1.19</td>
<td>1.43</td>
<td>0.02</td>
</tr>
<tr>
<td>95%CI</td>
<td>(0.72 - 1.37)</td>
<td>(0.76 - 1.42)</td>
<td>(0.87 - 1.62)</td>
<td>(1.03 - 1.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P†</td>
<td>1.0</td>
<td>0.8</td>
<td>0.3</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios calculated by conditional logistic regression, taking into account matching for age, sex, and enrolment time. P* indicates p-value for linearity between CETP quintiles and CAD risk. Below the odds ratios, corresponding 95% confidence intervals and p-values (P†) are presented for that quintile. The odds ratios for CAD risk were adjusted for smoking (never, past, current), diabetes, systolic blood pressure, body mass index, CRP levels, and fasting time.

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 to those in the lowest quintile (OR = 1.43, 95%CI = 1.03-1.99, 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 mg/l 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 mg/l 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 1). In contrast, CAD risk did increase among individuals with high triglyceride levels (p for linearity = 0.02). Compared to individuals in the lowest quintile, those in the second, third, fourth and fifth quintile had the following respective ORs for future CAD: 1.50 (95%CI = 0.84-2.67), 1.51 (95%CI = 0.84-2.69), 1.84 (95%CI = 1.02-3.32), and 1.87 (95%CI = 1.06-3.30) (figure 1). 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 borderline statistical significance when CETP levels were entered as a continuous variable (p = 0.047).
Upon 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 = 1.02-3.68, p = 0.045 for people in the highest CETP quintile compared to those in the lowest one). However, upon additional adjustment for HDL-c levels and upon additional adjustment for the ratio LDL-c / HDL-c, the relationship between CETP levels and CAD risk was attenuated and became non-significant; OR = 1.64 (95%CI = 0.86-3.13, p = 0.1) and OR = 1.60 (95%CI = 0.82-3.11, p = 0.2), respectively for those in the highest CETP quintile compared to 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 non-lipid 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, i.e. 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 that were selected on the basis of abnormal lipid levels, lipid disorders, diabetes, or CAD. In contrast, the current 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, were also 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 the risk of coronary artery disease
The current study shows that increasing CETP levels are associated with an increased risk of future CAD in apparently healthy individuals. To our knowledge, only one small cross-sectional study in Chinese has also investigated the relationship between CETP plasma levels and cardiovascular endpoints. In line with our observations, these investigators reported that CETP plasma levels were higher in a group of patients who suffered from myocardial infarction than in healthy controls.\(^{34}\) Two other
**Figure 1.** Odds ratios for future CAD according to CETP quintile for individuals below and above the median triglyceride concentration in EPIC-Norfolk 1993-2003.

Odds ratios and corresponding 95% confidence intervals calculated by conditional logistic regression, taking into account matching for age, sex, and enrolment time. Odds ratios were adjusted for smoking (never, past, current), diabetes, systolic blood pressure, body mass index, and CRP levels. Grey diamonds represent individuals with triglyceride levels below 1.7 mmol/l (p for linearity = 0.5), and black squares represent those with triglyceride levels above the median (p for linearity = 0.02).

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 to those with low CETP levels.\(^{29}\) We have also used B-mode ultrasound to measure the intima media thickness (IMT) of the carotid artery in patients with familial hypercholesterolemia and have shown again that elevated CETP levels were associated with increased progression of atherosclerosis.\(^{28}\) In addition to studies among healthy individuals and patients with CAD, studies in subjects with genetic CETP deficiency have also provided insight in 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{29}

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 pre-defined and was driven by the evidence that CETP-facilitated transfer of cholesteryl esters 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 are 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 our study merit further consideration. First, plasma levels of CETP were determined in a non-fasting 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 current 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 be not be consistent with the 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 concerning the potential effects of currently tested CETP inhibitors. First of all, we have studied apparently healthy individuals while 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 upon pharmacological inhibition which is accompanied by an increase in CETP plasma concentration probably because the interaction of the small molecules with the CETP protein disturbed CETP clearance from the circulation.\textsuperscript{10,11} Taking these considerations 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 as well as clinical endpoints will have to answer this question.
Conclusion
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 = 1.06-3.30, p = 0.02), compared to 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.

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References


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