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High plasma cholesteryl ester transfer protein levels may favour reduced incidence of cardiovascular events in men with low triglycerides

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Aims High cholesteryl ester transfer protein (CETP) concentrations are associated with increased risk of cardiovascular disease (CVD) in subjects with high triglycerides. We determined the relationship of plasma CETP with incident CVD in a population with relatively low triglycerides.
Methods and results A nested case–control study was performed in men participating in the prospective PREVEND study, after exclusion of CVD, diabetes mellitus, and lipid-lowering drugs use at baseline. Plasma CETP was measured in 111 men who developed a cardiovascular event (cases) during follow-up and in 116 controls who remained free of CVD. Fasting total cholesterol ($P < 0.001$) and triglycerides ($P < 0.001$) were higher, HDL cholesterol was lower ($P = 0.001$), but CETP was similar in cases and controls ($P = 0.39$). Cox proportional hazards regression analysis showed that CVD risk tended to be lower with higher plasma CETP after adjustment for age and lipids (hazard ratio 0.84; 95% CI 0.69–1.03, $P = 0.10$). Plasma CETP was lower in cases than in controls ($P = 0.05$) with triglycerides $\leq$1.38 mmol/L (median), but not with higher triglycerides. The age-adjusted hazard ratio for CVD was 0.46 (95% CI 0.24–0.90) in men with triglycerides $\leq$1.38 mmol/L and CETP >2.26 mg/L (median) compared with men with similarly low triglycerides and CETP $\leq$2.26 mg/L. With higher triglycerides, the hazard ratio for CVD was similar in both CETP categories.
Conclusion Relatively high plasma CETP may favour reduced CVD risk in the context of low triglycerides.

Introduction

Cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL metabolism in humans, as evidenced by the markedly elevated HDL cholesterol levels in subjects with genetic CETP deficiency[1–3] and the strong increase in HDL cholesterol in response to pharmacological CETP inhibition.[4,5] This lipid transfer protein enables the transfer of cholesteryl esters from HDL towards very low and low-density lipoproteins (VLDL and LDL), whereas triglycerides are transferred in the opposite direction.[6,7] As a result of CETP action, HDL cholesterol is decreased, the cholesterol content in VLDL is increased, and the generation of small dense LDL particles is enhanced, particularly in hypertriglyceridaemia.[7–10]

Despite intensive research, the potential impact of CETP on cardiovascular disease (CVD) is still debated. On the one hand, the CETP-mediated cholesteryl ester transfer process contributes to an atherogenic lipoprotein profile, and species which naturally lack CETP, such as rats, are relatively resistant to diet-induced atherosclerosis.[11] On the other hand, the cholesteryl ester transfer process provides an additional route for delivery of HDL-derived cholesteryl esters to the liver via VLDL and LDL.[7,9,10,12–14] Moreover, intracellular CETP could directly promote cholesterol removal from peripheral cells,[15] and circulating CETP is involved in the generation of small, lipid, poor pre-β-HDL particles that act as initial acceptors of cell-derived cholesterol.[6,14,16–18] In addition, CETP is present in hepatocytes and may directly stimulate hepatic uptake of cholesteryl esters from HDL.[19] Thus, CETP may beneficially affect reverse cholesterol transport, i.e. the pathway whereby

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cholesterol is removed from peripheral cells to the liver, where it is metabolized and excreted in the bile.[6,10,14]

Importantly, one prospective case–control study has shown that the plasma CETP concentration is a predictor of CVD but only in subjects with high plasma triglycerides.[20] We have recently observed that the rate of plasma cholesteryl ester transfer, rather than the plasma CETP concentration *per se*, is an independent determinant of intima media thickness, a well-accepted marker of subclinical atherosclerosis.[21] Since plasma cholesteryl ester transfer is determined by both the plasma triglyceride level, reflecting the constellation of VLDL particles that accept cholesteryl esters from HDL, and the CETP concentration, these findings are consistent with the notion that CETP may indirectly increase cardiovascular risk in the context of higher triglyceride levels. Collectively, these findings strongly suggest that the plasma triglyceride level modifies the effect of circulating CETP on cardiovascular risk.

The primary objective of our study was to determine the impact of plasma CETP on incident CVD, in a population with relative low triglycerides.[22] As a secondary objective, we determined the relation of CETP with cardiovascular risk in the context of relatively lower and higher triglyceride levels. To this end, we carried out a prospective nested case–control study among men participating in the PREVEND (Prevention of REnal and Vascular ENd stage Disease) project.

Methods

Study design and participants

We performed a nested case–control study among subjects participating in the PREVEND cohort. This study investigates vascular and renal damage in a predominantly Caucasian population. Details of the study have been described elsewhere.[23] In summary, in 1997–98, all inhabitants of the city of Groningen, aged 28–75 years, were sent a short questionnaire on demographics and cardiovascular morbidity and a vial to collect an early morning urine sample. Altogether, 40 856 subjects responded (47.8%). Pregnant women and diabetic subjects using insulin were excluded. All participants with urinary albumin concentration >10 mg/L were invited to our clinic together with randomly selected subjects with a urinary albumin concentration <10 mg/L. So, the PREVEND Study consists of a population enriched with subjects with microalbuminuria. The study population comprised 8592 subjects who completed the total screening programme. The study was approved by the local medical Ethics Committee. All participants gave written informed consent.

Both HDL cholesterol and the plasma CETP levels have been reported to be lower in men than in women.[24,25] This strongly suggest that the effect of CETP action on HDL differs between sexes, making it necessary to evaluate the effect of plasma CETP on CVD in men and women separately. Since it was anticipated that the event rate was low in women, we decided to carry out the present study in men only. Only men with a negative history of myocardial infarction (MI), major ischaemia, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass graft (CABG) at baseline were selected. Information regarding the use of antihypertensive and blood glucose- and lipid-lowering drugs, smoking, and alcohol consumption (categorized as <1 and ≥ 1 U/day) was obtained using a checklist as described.[23] Body mass index (BMI) was calculated as the ratio between weight and height squared (in kg/m^2). Waist circumference was measured on bare skin between the tenth rib and the iliac crest. Hypertension was characterized as systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg[26] or the use of antihypertensive drugs. Men with diabetes mellitus, defined as fasting plasma glucose $\geq$7.0 mmol/L or the use of glucose-lowering agents, the use of lipid-lowering drugs, and with urinary albumin excretion >300 μg/min, were excluded.

The combined endpoint of our study was incident CVD, defined as death from CVD (ICD-10 I00–I02, I05–I15, I20–I28, I30–I52, I60–I89, I95–I99), hospitalization for MI (ICD-10 I21, I22), PTCA, or CABG. From the time of inclusion in the study, the vital status of the participants was checked through the municipal register. The cause of death was obtained by linking the number of the death certificates to the primary cause of death as coded by a physician from the Central Bureau of Statistics (CBS, Voorburg/Heerlen, The Netherlands). Causes of death were coded according to the 10th revision of the International Classification of Diseases (ICD-10). Information on MI, PTCA, and CABG was obtained from national hospital information system (Prismant, Utrecht, The Netherlands). Cases were men who had an event in the period from the date of the outpatient clinic baseline assessment to 31 December 2003, or 31 December 2002 until which date information regarding specific causes of death follow-up information was available.

In total, 129 men who had an event until census date were identified. An equal number of male control subjects were randomly selected from the whole baseline study cohort after exclusion of those men who developed cardiovascular event. Baseline plasma samples of sufficient quality for CETP measurement from 111 out of 129 cases, and from 116 out of 129 control subjects were available. There were no differences in clinical characteristics as well as in plasma lipids and apolipoproteins (apos) between the men whose plasma CETP level was available ($n = 227$) compared with the men in whom CETP was lacking ($n = 31$, data not shown), except that men without information on CETP were older (61.9 ± 10.5 vs. 52.5 ± 12.2 years, $P < 0.001$) and had a lower urinary albumin excretion [5.2 (4.4–13.9) vs. 13.6 (7.9–23.8) μg/min, $P < 0.001$].

Laboratory methods

Blood samples were taken after 15 min rest. Plasma glucose was measured shortly after blood sampling. Plasma was obtained by centrifugation at 4°C, and the samples were stored at -80°C until analysis. HDL cholesterol was measured with a homogeneous method (direct HDL, AEROSET™ System, Abbott Laboratories, Abbott Park, USA).[27] Triglycerides were measured enzymatically. Total cholesterol and plasma glucose were assessed using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York, USA). Apo A-I and apo B were determined by nephelometry applying commercially available reagents for Dade Behring nephelometer systems (BN II, Dade Behring Marburg, Germany).[28,29] Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany). The mean of two 24 h urine collections was used to determine urinary albumin excretion.

Plasma CETP concentration was analysed using a double-antibody sandwich ELISA.[30] A combination of monoclonal antibodies TP1 and TP2 was employed as coating antibodies and monoclonal antibody TP20, labelled with digoxigenine, as the secondary antibody. CETP control samples were validated by R.M. McPherson, Montreal, Canada, using a radioimmunoassay.

Statistical analyses

Stata SE 8, SPSS 12, and Excel were used for data analysis. Data are expressed as mean $\pm$ SD or in median (interquartile range). Between-group differences were compared with Student's *t*-test and by Mann–Whitney analyses, where appropriate. χ^2 analysis was used to compare frequencies between groups. A list of factors were created, which could be associated with the exposure (CETP concentration) under study, and a list was made of factors which were likely to be associated with the outcome. For many potential confounders, an association with CETP and the outcome could not be established. An association between CETP concentration and

potential confounders in this study was only suggested regarding lipid parameters (not shown). The relation between CETP and potential confounders and the incidence of CVD was analysed with the use of Cox proportional hazards models. Hazard ratios for continuous risk factors were standardized, thus expressing the risk associated with a 1-SD increase in the continuous risk factors. All Cox proportional hazards models were age or mutually adjusted. The assumption of proportionality of hazards was checked by means of Schoenfeld residuals, using procedure 'stphtest' in STATA, which is based on the methods described by Grambsch and Therneau. No severe deviations from parallelism were evident. The assumption of linearity was checked graphically by studying the smoothed martingal residuals from the null model plotted against the covariate variables. The linearity assumptions were satisfied. In a secondary analysis, the subjects were also grouped according to the median triglyceride and the median CETP level to determine the effect of CETP on CVD risk in the context of lower and higher triglycerides. In these models, hazard ratios were adjusted for age. Since our cohort consisted of a random sample of subjects with <10 mg/L of urinary albumin concentration and all subjects with >10 mg/L of urinary albumin, risk estimates were also calculated by adding the selection parameter as an effect modifier interacting with the variables for CETP and plasma lipids. A two-sided *P*-value ≤ 0.05 was considered to be significant.

Results

Median follow-up was 1021 (interquartile range 581–1579) days in men who developed CVD (cases) and 2020 (1930–2113) days in men who remained free of CVD (controls). *Table 1* shows clinical and laboratory characteristics of 111 cases and 116 controls. Cases were older and had a higher BMI, waist circumference, and urinary albumin excretion. There were more smokers among men with incident CVD and they had hypertension more frequently. Total cholesterol, non-HDL cholesterol, triglycerides, and apo B-levels were higher, whereas HDL cholesterol and apo A-I were lower in cases than in controls. Plasma CETP levels were not significantly different between the two groups.

As shown in *Table 2*, Cox proportional hazards regression analysis with plasma lipids and CETP levels showed that plasma total cholesterol, HDL cholesterol, and triglycerides were significant determinants of age-adjusted incident CVD. In a mutually adjusted model, age-adjusted incident CVD was also independently determined by HDL cholesterol and plasma triglycerides, although there was now a trend for lower risk with higher CETP levels.

To determine whether the plasma CETP level influences CVD risk in the context of lower or higher triglycerides, we compared plasma CETP between men who remained free of CVD and men who developed CVD, stratified according to the median triglyceride level (1.38 mmol/L) of the whole study population ($n = 227$). In men with triglycerides ≤ 1.38 mmol/L, plasma CETP was lower in men who developed CVD (2.10 ± 0.76 mg/L) than in men who remained free of CVD (2.40 ± 0.79 mg/L, $P = 0.05$). In contrast, in men with triglycerides >1.38 mmol/L, there was no difference in CETP between cases (2.49 ± 0.90 mg/L) and controls (2.47 ± 0.81 mg/L, $P = 0.89$).

To further evaluate the effect of CETP on CVD risk, the study population was divided in four groups according to the median triglyceride level and the median plasma CETP level of the entire study population (2.26 mg/L). As shown in *Table 3*, the percentage of subjects who developed CVD was lower in men with lower triglyceride and higher CETP levels compared with men with lower triglyceride and lower CETP levels, but there were no significant differences in any clinical characteristics and plasma (apo)lipoproteins between these subgroups. There were, however, no differences in the percentage of men who developed CVD, nor in clinical characteristics and plasma (apo)lipoprotein levels in the subgroup with higher triglyceride and higher CETP levels compared with the subgroup with higher triglyceride and lower CETP levels (*Table 3*). Men with triglycerides >1.38 mmol/L had higher total cholesterol, non-HDL cholesterol and apo B, as well as lower HDL cholesterol and apo A-I levels ($P \leq 0.001$ for all), but plasma CETP mass was not significantly different ($n = 113$, 2.47 ± 0.84 mg/L) compared with men with triglycerides ≤ 1.38 mmol/L ($n = 114$, 2.30 ± 0.79 mg/L, $P = 0.10$).

As shown in *Table 4*, at lower plasma triglyceride levels, the age-adjusted hazard ratio for incident CVD was decreased to 0.46 (95% CI 0.24–0.90, $P = 0.03$) in men with higher CETP levels compared with men with lower CETP. After additional adjustment for smoking, urinary albumin excretion, hypertension, and the use of alcohol, this hazard ratio was 0.49 (95% CI 0.25–0.97, $P = 0.04$). In contrast, no significant effect of the CETP level on incident CVD was observed in men with triglycerides above the median value (HR 1.00, 95% CI 0.63–1.58, $P = 0.98$).

Table 1 Baseline clinical characteristics, plasma lipids, apolipoproteins (apos), and cholesteryl ester transfer protein (CETP) mass in men who developed cardiovascular disease (CVD) (cases) and in men who remained free of CVD (controls)

	Cases ($n = 111$)	Controls ($n = 116$)	*P*
Age (years)	58.0 ± 10.3	47.2 ± 11.5	<0.001
BMI (kg/m^2)	27.5 ± 3.9	25.7 ± 3.1	<0.001
Waist (cm)	97.9 ± 10.6	92.0 ± 9.4	<0.001
Cigarette smokers (%)	55.0	37.1	0.01
Alcohol users (>1 per day, %)	65.8	73.9	0.18
Hypertension (%)	55.5	30.7	<0.001
Urinary albumin excretion (μg/min)	18.1 (9.7–36.3)	9.5 (6.4–17.2)	<0.001
Total cholesterol (mmol/L)	6.25 ± 1.12	5.60 ± 1.04	<0.001
Non-HDL cholesterol (mmol/L)	5.20 ± 1.18	4.39 ± 1.10	<0.001
HDL cholesterol (mmol/L)	1.05 ± 0.35	1.20 ± 0.34	0.001
Triglycerides (mmol/L)	1.61 (1.11–2.38)	1.17 (0.84–1.64)	<0.001
Apo B (g/L)	1.24 ± 0.38	1.07 ± 0.26	<0.001
Apo A-I (g/L)	1.24 ± 0.28	1.33 ± 0.22	0.01
CETP mass (mg/L)	2.34 ± 0.80	2.43 ± 0.82	0.39

Table 2 Effect of plasma lipids and cholesteryl ester transfer protein concentration on incident cardiovascular disease by Cox proportional hazard regression analysis

	HR (95% CI) (age-adjusted)	*P*	HR (95% CI) (mutually adjusted)	*P*
Total cholesterol	1.26 (1.04–1.52)	0.20	1.18 (0.96–1.46)	0.12
HDL cholesterol	0.66 (0.53–0.82)	<0.001	0.71 (0.54–0.92)	0.01
Ln triglycerides	1.43 (1.19–1.72)	<0.001	1.16 (0.90–1.48)	0.26
CETP	0.88 (0.73–1.07)	0.21	0.84 (0.69–1.03)	0.10

Hazard ratios (HRs) are given per 1 SD increase in plasma lipids and cholesteryl ester transfer protein values. Left column shows HRs adjusted for age only. Right column shows mutually, including age, adjusted HRs.

We also analysed the data making use of another strategy of dichotomizing the distribution of plasma CETP and triglycerides, i.e. the highest quartile of CETP vs. a combination of the three lowest quartiles of CETP and the lowest quartile of triglycerides vs. the highest quartiles of triglycerides, as well as by stratifying below and above a plasma triglyceride level of 1.7 mmol/L, which was the median value in the EPIC-Norfolk study.[20] Both age-adjusted analyses showed similar trends that the hazard ratio was lowest in the subgroup with high CETP and low triglyceride levels compared with low triglycerides and low CETP; HR 0.30 (95% CI 0.07–1.31, $P = 0.11$) and HR 0.61 (95% CI 0.37–1.04, $P = 0.07$), respectively. Furthermore, we also conducted an analysis, using plasma CETP and plasma triglycerides as continuous variables, and calculated the interaction term. In this analysis, the interaction between CETP and triglycerides was not significant ($P = 0.21$, age-adjusted).

Finally, it was explored whether the preferential inclusion of subjects with elevated levels of urinary albumin concentration in the PREVEND cohort affected the relationship between plasma lipids and CETP levels on incident CVD. The procedure for participating in PREVEND did not materially alter the results, and no significant interaction was observed.

Discussion

The present prospective nested case–control study is the first that is focused on the association of circulating CETP concentration with incident CVD in a population with relatively low fasting plasma triglycerides. Plasma CETP mass was not different in cases compared with control subjects, and Cox proportional hazards regression analysis demonstrated that cardiovascular risk tended to be lower with higher CETP levels after adjustment for age and lipid parameters. Thus, our study shows that a higher plasma CETP level is not associated with increased cardiovascular risk in men with relatively low triglycerides. Further analysis suggested that in men with triglyceride levels below the median value of the whole study group, a high plasma CETP level was associated with a reduced incidence of CVD compared with the risk observed in subjects with similarly low triglycerides but low CETP mass levels. Among men with lower triglycerides, the association of high plasma CETP with reduced CVD risk at lower could not be explained by differences in clinical characteristics, nor by differences in plasma lipid levels between men with higher compared with lower CETP levels. These findings remained after adjustment for well-accepted clinical cardiovascular risk factors such as smoking, hypertension, urinary albumin excretion, as well as alcohol consumption.

Median fasting plasma triglycerides amount to 1.14 mmol/L in the whole PREVEND population,[22] a value which closely corresponds with the level of 1.17 mmol/L in the presently studied control subjects. In a meta-analysis of surveys in which the effect of HDL cholesterol and CETP gene variation on cardiovascular risk was determined, the median triglyceride level was reported to vary between 0.9 and 1.7 mmol/L across populations.[31] This underscores that plasma triglycerides are relatively low in the PREVEND cohort, but it is also evident that such a degree of triglyceridaemia is not unique in comparison with other cohorts. In the EPIC-Norfolk study, the detrimental effect of plasma CETP on cardiovascular risk was limited to those subjects in whom plasma triglycerides (in part of the population measured in the non-fasting state) were >1.7 mmol/L.[20] In the present study, the median triglyceride level was 1.38 mmol/L in cases and control subjects combined. In view of this difference in triglycerides between the present report and the EPIC-Norfolk study, we consider our results to be complementary rather than contradictory with that survey.[20] Furthermore, close inspection of the graphical data from the EPIC-Norfolk study suggests that there may indeed have been a tendency towards a reduced cardiovascular risk in association with a high CETP mass in subjects with triglycerides <1.7 mmol/L. In our study, the hazard for incident CVD was not increased in men with higher triglycerides and higher CETP levels compared with men with higher triglycerides and lower CETP. This lack of association of CVD risk with circulating CETP at triglycerides above the median value of the whole study population could again be attributable to relatively low triglyceride levels (median, 2.0 mmol/L) even in those subjects, but also to insufficient statistical power. Considering the EPIC-Norfolk study and our data together, it is tempting to speculate that there may be a U-shaped relationship between circulating CETP and CVD risk such that there is a beneficial effect of CETP on CVD at low triglycerides, which disappears at intermediate triglyceride levels and changes into a deleterious effect at high triglycerides.

In view of the multiple roles of CETP in lipoprotein metabolism, there is an ongoing debate about the putative pro- or anti-atherogenic effects of circulating CETP in humans.[32–34] Our population-based study suggests that high plasma CETP levels could protect against CVD in men with the lowest triglyceride levels. Among other possible mechanisms, this potentially beneficial effect of CETP at low triglycerides

Table 3 Incidence of cardiovascular disease, baseline clinical characteristics, plasma lipids and apos, and cholesteryl ester transfer protein mass according to plasma triglycerides (TG) and cholesteryl ester transfer protein

	Subgroup 1 TG $\leq$ 1.38 mmol/L, CETP $<$ 2.26 mg/L, $n = 58$	Subgroup 2 TG $\leq$ 1.38 mmol/L, CETP $>$ 2.26 mg/L, $n = 55$	*P* of difference	Subgroup 3 TG $>$ 1.38 mmol/L, CETP $<$ 2.26 mg/L, $n = 56$	Subgroup 4 TG $>$ 1.38 mmol/L, CETP $>$ 2.26 mg/L, $n = 58$	*P* of difference
Incident CVD (percentage of subjects)	44.1	23.6	0.02	63.6	63.8	0.99
Age (years)	52.2 $\pm$ 14.0	50.4 $\pm$ 12.7	0.47	53.9 $\pm$ 10.5	53.4 $\pm$ 11.1	0.83
BMI (kg/m^2)	25.9 $\pm$ 3.9	25.1 $\pm$ 2.5	0.21	27.8 $\pm$ 4.0	27.5 $\pm$ 3.2	0.71
Waist (cm)	92.5 $\pm$ 10.9	90.7 $\pm$ 8.4	0.33	99.3 $\pm$ 10.5	97.2 $\pm$ 9.5	0.25
Cigarette smokers (%)	39.0	40.0	0.91	56.4	48.3	0.39
Alcohol users ($>$1 unit per day, %)	79.3	69.1	0.21	67.3	63.8	0.70
Hypertension (%)	33.9	38.2	0.63	49.1	50.9	0.85
Urinary albumin excretion (μg/min)	12.2 (7.3–23.8)	9.7 (6.4–15.8)	0.15	15.0 (9.7–31.0)	16.9 (8.0–36.2)	0.91
Total cholesterol (mmol/L)	5.27 $\pm$ 0.91	5.55 $\pm$ 1.02	0.13	6.37 $\pm$ 1.07	6.49 $\pm$ 1.02	0.56
Non-HDL cholesterol (mmol/L)	4.00 $\pm$ 0.92	4.26 $\pm$ 1.04	0.16	5.35 $\pm$ 1.05	5.55 $\pm$ 1.03	0.31
HDL cholesterol (mmol/L)	1.27 $\pm$ 0.36	1.29 $\pm$ 0.37	0.77	1.02 $\pm$ 0.26	0.94 $\pm$ 0.26	0.10
Triglycerides (mmol/L)	0.96 (0.79–1.14)	0.98 (0.77–1.17)	0.89	1.88 (1.61–2.54)	2.10 (1.63–2.80)	0.36
Apo B (g/L)	0.96 $\pm$ 0.20	1.04 $\pm$ 0.25	0.06	1.27 $\pm$ 0.33	1.33 $\pm$ 0.37	0.42
Apo A-I (g/L)	1.33 $\pm$ 0.25	1.37 $\pm$ 0.23	0.42	1.22 $\pm$ 0.29	1.22 $\pm$ 0.20	0.89
CETP (mg/L)	1.69 $\pm$ 0.33	2.95 $\pm$ 0.58	$<$0.001	1.83 $\pm$ 0.32	3.09 $\pm$ 0.70	$<$0.001

Table 4 Age-adjusted HRs for incident cardiovascular disease according to baseline triglycerides and cholesteryl ester transfer protein mass

	A		B		C	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Subgroup 1	1					
Subgroup 2	0.46 (0.24–0.90)	0.03	1			
Subgroup 3	1.39 (0.84–2.31)	0.20			1	
Subgroup 4	1.38 (0.84–2.29)	0.21	3.00 (1.59–5.64)	0.001	1.00 (0.63–1.58)	0.98

A, B, C: Hazard ratios calculated with group 1, 2, and 3 as reference group, respectively.
Subgroup 1: TG≤1.38 mmol/L, CETP<2.26 mg/L; subgroup 2: TG≤1.38 mmol/L, CETP>2.26 mg/L; subgroup 3: TG>1.38 mmol/L, CETP<2.26 mg/L; subgroup 4: TG>1.38 mmol/L, CETP>2.26 mg/L.

may be explained by assuming that a certain rate of cholesteryl ester transfer from HDL towards VLDL and LDL is necessary for an effective reverse cholesterol transport pathway.[32] In this regard, it is important that in normolipidaemic subjects, the major route for delivery of HDL-derived cholesteryl esters to the liver is via apo B-containing lipoproteins, whereas there is little net transport of cholesteryl esters to the liver directly via HDL.[13] These kinetic data support the notion that the CETP-mediated cholesteryl ester transfer process substantially contributes to the reverse cholesterol transport pathway in humans *in vivo*. Moreover, although equivocal results have been reported concerning the association of common CETP gene variations with cardiovascular risk,[31,35,36] (partial) genetic CETP deficiency is reported to be related to increased cardiovascular risk in the context of intermediate HDL cholesterol levels.[2]

Certain methodological aspects of our study need to be considered. Firstly, in order to avoid heterogeneity in cardiovascular endpoints as much as possible, we decided to include only subjects who died from CVDs, as well as subjects who suffered from proven MI or in whom coronary intervention was performed during follow-up. Consequently, the number of cases was rather low in the present survey. Secondly, we included only men (for reasons, see **Methods** section). Therefore, our results cannot be extrapolated to women. Thirdly, this study was performed in a cohort in which subjects with elevated urinary albumin excretion were preferentially included. However, this is very unlikely to have affected the interpretation of our results because neither controlling for our study design nor adjustment for urinary albumin excretion did have any effect on the reduced hazard ratio for CVD observed in men with lower triglycerides and higher CETP levels. Finally, the division of the subjects in subgroups according to median plasma triglyceride and CETP mass levels of the entire study population is somewhat arbitrary. Using plasma CETP and plasma triglycerides as continuous variables, a non-significant interaction term was found. However, also when plasma CETP and triglycerides were dichotomized using combinations of different quartiles and when dichotomized using the median value of triglyceride levels as found in the EPIC-Norfolk study, similar results were observed.[20] Our findings need to be confirmed in further prospective studies. Moreover, the cut-off levels presented in this study should not be interpreted in absolute terms.

The present findings may have practical implications. Firstly, our results raise the possibility that measurement of plasma CETP mass could be valuable for cardiovascular risk assessment in men with low triglycerides. Secondly, our results would suggest that CETP inhibitor treatment is not beneficial in subjects with low triglycerides and high CETP levels.

In conclusion, this study supports the hypothesis that the plasma CETP level can modulate the effect of triglycerides on cardiovascular risk. In men with the lowest triglycerides, a relatively high CETP level may favour reduced cardiovascular risk.

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Conflict of interest: none declared.

Appendix

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