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Elevated cholesteryl ester transfer protein concentration is associated with an increased risk for cardiovascular disease in women, but not in men, with Type 2 diabetes: the Hoorn Study

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Abstract

Aims Cholesteryl ester transfer protein (CETP) exchanges neutral lipids between lipoproteins. As the role of CETP in the atherogenic process is still not fully clarified, we studied the association of CETP concentration with the prevalence of cardiovascular disease (CVD) and with intima-media thickness of the carotid artery (IMT) in subjects with normal glucose tolerance (NGT), impaird fasting glucose and/or impaired glucose tolerance (IFG/IGT) and Type 2 diabetes mellitus.

Methods Subjects (n = 566) were recruited from the 2000–2001 follow-up examination of the Hoorn study. CETP concentration was determined by immunoassay. CVD was defined as self-reported history of arterial surgery, cerebral vascular event, amputation, angina, claudication, possible infarction, measured ankle-brachial index < 0.90 or ECG abnormalities. The right common carotid artery IMT was measured by ultrasound at 10 mm proximal to the carotid bulb.

Results In men, CETP concentration was not associated with CVD, irrespective of glucose tolerance status. In women with NGT or IGT, there was also no relationship. However, in women with Type 2 diabetes, the risk of CVD was increased in those with high CETP concentration [odds ratio = 3.34 (1.56; 7.14)]. No statistically significant association was found between CETP concentration and IMT in the entire cohort.

Conclusions In an elderly Caucasian population, associations of CETP concentration with CVD were dependent on glucose tolerance status and gender. The finding that high CETP concentration was strongly associated with increased prevalence of CVD in women with Type 2 diabetes warrants further investigation.

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Keywords cardiovascular disease, intima-media thickness of the carotid artery, lipoproteins, Type 2 diabetes

Abbreviations BMI, body mass index; CE, cholesterol ester; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; HDL-c, HDL cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IMT, intima-media thickness; LDL, low-density lipoprotein; LDL-c, LDL cholesterol; NGT, normal glucose tolerance; OR, odds ratio; VLDL, very low-density lipoprotein

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Introduction

Disturbances in lipoprotein metabolism play a major role in atherogenesis. Increased levels of low-density lipoprotein (LDL) cholesterol (LDL-c) and triglycerides, associated with the occurrence of small dense LDL, and decreased levels of HDL-cholesterol (HDL-c) characterize dyslipidaemia. Cholesteryl ester transfer protein (CETP) plays an important role in lipid metabolism by mediating the transfer of esterified cholesterol (CE) from high-density lipoprotein (HDL) to apolipoprotein B-containing lipoproteins in exchange for triglycerides [1].

To date, it has been difficult to define the role of CETP in atherogenesis. A high CETP activity decreases HDL-c levels and lowering CETP activity by investigational drugs has been shown to increase HDL-c concentration [2] and to decrease LDL-c concentration [3]. In contrast, CETP plays an important role in reverse cholesterol transport by promoting the transport of CE to the liver by LDL or very low-density lipoprotein (VLDL) [4]. In the presence of efficient hepatic uptake of the latter lipoproteins or their contents, this may be favourable. It is likely that the individual lipid profile determines the way in which CETP affects atherogenesis [5]. In a number of previous studies, high CETP concentration was associated with increased risk of coronary artery disease in individuals with increased triglyceride levels [6], familial hypercholesterolaemia [7], men with established coronary artery disease [8] and in Type 2 diabetic patients [9]. One study showed that the association between CETP and coronary artery disease was explained by decreased HDL-c levels [6], while other studies did not [7–9].

Disturbances in lipid metabolism and high risk of atherosclerosis and cardiovascular disease (CVD) are common features of diabetes. Little is known about the association between CETP concentration and CVD in these patients. Therefore, we studied the associations of CETP concentration with CVD and intima-media thickness (IMT) of the carotid artery in subjects with normal glucose tolerance (NGT), impaired fasting glucose and/or impaired glucose tolerance (IFG/IGT) and Type 2 diabetes mellitus.

In addition, we investigated the association of the −629C/A polymorphism in the promotor region of the CETP gene with CVD and IMT.

Subjects and methods

Study population

The Hoorn Study is a population-based cohort study of Type 2 diabetes in the general Dutch population. The population and the study design have been described in detail previously [10]. Briefly, in 1989, 3553 men and women, aged 50–74 years, were randomly selected from the population register of the medium-sized Dutch town of Hoorn; 2484 Caucasian subjects agreed to participate. In the 2000–2001 Hoorn Study follow-up examination, 1074 individuals of the original Hoorn Study cohort were re-invited. We invited all surviving subjects with Type 2 diabetes (n = 176), and random samples of individuals with normal glucose tolerance (n = 705) or impaired glucose tolerance (n = 193) based on their glucose tolerance status at the previous examination (WHO-99 criteria) [11]. Of these 1074 subjects, 648 (60.3%) subjects participated in the follow-up examination. To increase the number of diabetic individuals, we additionally invited 217 participants from the Hoorn Screening Study [12], of whom 188 agreed to participate. Among the 455 non-participants, 13% were complete non-responders. Other reasons not to participate were lack of interest (30%), co-morbidity (23%), age (7%), unwillingness to travel (6%), participation too time-consuming (6%), and miscellaneous reasons (15%) [13]. Finally, 836 subjects participated in the study. The study was approved by the ethics committee of the VU University Medical Center. All subjects gave written informed consent.

From the participating 836 subjects, we excluded subjects with missing data on primary variables of interest [glucose tolerance status (n = 24), CETP concentration (n = 52), ultrasound examination of the carotid artery (n = 76) mainly because of unsatisfactory definition of the arterial wall because of obesity [13]]. Subjects who used medication which is known to affect lipid metabolism (n = 118) and CETP concentration [14] were also excluded. The resulting complete dataset for primary values of interest contained of 566 subjects (231 with NGT, 127 with IFG/IGT and 208 with Type 2 diabetes). Glucose tolerance status was defined again at the follow-up examination according to the WHO-99 criteria [11].

Anthropometric measurements and questionnaires

Anthropometric measurements were obtained from all participants. Weight and height were measured in barefooted participants wearing light clothes only. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Information about alcohol intake was obtained from a validated food-frequency questionnaire [15].

Laboratory analyses

Fasting and 2-h post-load plasma glucose concentrations were determined by the glucose dehydrogenase method and triglycerides, total cholesterol and HDL-c were determined by enzymatic techniques (Roche, Mannheim, Germany).

LDL-c was directly determined by the N-geneous™ assay (Genzyme, Cambridge, MA, USA). LDL size was measured by high-performance gel-filtration chromatography as previously described in detail [16,17].

CETP concentration was measured by a two-antibody sandwich immunoassay, which was developed and described by Niemeijer-Kanters *et al.* [18]. Inter-assay and intra-assay coefficients of variation were 7.8 and 6.0%, respectively. As a standard, pool plasma containing 2 mg CETP/mL was taken. DNA was extracted from white blood cells for genotyping. We used the polymerase chain reaction method as described by Klerkx and co-workers to assess the presence of the C→A variance in the promoter region of the CETP gene [19].

Cardiovascular disease

Resting 12-lead ECGs were recorded and automatically coded according to the Minnesota code [20] with an automated

diagnostic classification system (Modular ECG Analysis System) [21]. An ECG was considered abnormal with Minnesota codes 1–1, 1–2, 1–3, 4–1, 4–2, 4–3, 5–1, 5–2, 5–3 or 7–1.

Doppler-assisted systolic blood pressure measurements were performed in duplicate, from brachial, posterior tibial and dorsalis pedis arteries on both the left and the right side. Ankle-brachial index was calculated for each leg by the highest of the posterior tibial and the dorsalis pedis average pressure divided by the brachial pressure on the same side.

The Rose questionnaire [22] was used to determine presence of angina, claudication or possible myocardial infarction. CVD was defined as self-reported history of arterial surgery, cerebrovascular event, amputation, angina, claudication or possible infarction or measured ankle-brachial index < 0.90 or ECG abnormalities.

Ultrasound imaging

Procedures and reproducibility of scanning are described in detail elsewhere [13]. In summary, an ultrasound scanner (350 Series; Pie Medical, Maastricht, the Netherlands), equipped with a 7.5-MHz linear probe, was operated by a single observer. Three measurements, 4 s each, were performed in the right common carotid artery at 10 mm proximal to the carotid bulb. Images were registered and analysed by a computer equipped with vessel wall movement detection software and an acquisition system (Wall Track System; Pie Medical).

Statistical analyses

All analyses were performed in SPSS version 10.1 (SAS Institute, Cary, NC, USA). Characteristics of the study population were divided according to categories of glucose tolerance status and gender. Data are presented as means (SD) or for skewed data, as medians (interquartile range). Differences in baseline measurements between categories of glucose tolerance status and gender were examined using ANOVA for continuous data and Kruskal–Wallis analysis for dichotomous data. All ANOVA analyses were adjusted for age. Deviation from the Hardy–Weinberg equilibrium for frequencies of the –629C/A polymorphism was tested by chi-square.

Logistic regression was used to examine the association of CETP concentration with CVD. Age-adjusted odds ratios (OR) with 95% CI were obtained from logistic regression analysis. Linear regression analysis was used to examine the association of CETP concentration with IMT; results were reported as betas (95% CI). A beta of 10 would indicate that, if CETP concentration increases 1 mg/l, IMT increases by 10 μm. We tested for possible interactions of CETP concentration with glucose tolerance status, gender, smoking, triglycerides and study population (original Hoorn Study cohort or Hoorn Screening Study) by calculating the *P* values of the interaction terms.

Subsequently, we adjusted for HDL-c and triglycerides, which were considered as possible confounding factors. Finally, BMI, total cholesterol, systolic blood pressure, alcohol intake, smoking, LDL-c, LDL size, waist circumference and fasting insulin were considered as possible confounding factors and were added to a multivariate model. The final multivariate model included the factors that altered the estimated beta for prevalent CVD or IMT. For interaction, a *P* value < 0.10 was considered statistically significant, whereas we used *P* < 0.05 as statistically significant for all other analyses.

Results

Characteristics of the study population

Results were reported in groups of glucose tolerance status and for men and women separately because of observed interactions between CETP concentration and gender and between CETP concentration and glucose tolerance status with regard to CVD. Table 1 shows the characteristics of the study population stratified for categories of glucose tolerance status and gender. CETP concentration was higher in women than in men (*P* < 0.01, all men compared with all women), but did not differ significantly between categories of glucose tolerance. IMT was higher and CVD was commoner in subjects with IFG/IGT and diabetes in both sexes, although this increase only reached statistical significance in women (*P* < 0.01 and *P* = 0.01 for IMT and for CVD, respectively). The total prevalence of CVD was 48.9%. In the majority of these subjects, CVD was defined as ECG abnormalities, ankle-brachial index < 0.90, amputation or arterial surgery. The prevalence of self-reported CVD based on the Rose questionnaire only (angina, claudication and possible myocardial infarction) was 3% in the entire study population.

Associations of CETP concentration with CVD and IMT

In men, CETP concentration was not associated with the prevalence of CVD, independent of glucose tolerance status. In women with NGT or IFG/IGT, we also noted the absence of such a relation. However, in women with Type 2 diabetes, CETP concentration was strongly associated with CVD risk [OR = 3.34 (1.56; 7.14); *P* value for interaction between diabetes and CETP concentration: *P* < 0.01]. Importantly, this association was not attenuated after adjustments for HDL-c, triglycerides or other CVD risk factors (Table 2). Also, alcohol intake, LDL-c, LDL-size, waist circumference and fasting insulin did not alter the estimate (data not shown). In women with NGT, the weak inverse association between high CETP concentration and CVD was not statistically significant.

No association was found between CETP concentration and IMT in men with NGT, IFG/IGT or diabetes, nor was there an association in women with IFG/IGT or diabetes. The relationship between CETP concentration and IMT in women with NGT did not reach statistical significance (*P* = 0.054, Table 3). Because no statistically significant interaction with glucose tolerance status or gender was found with regard to IMT, a regression coefficient for CETP in the combined study population is considered more appropriate. CETP was not statistically significantly associated with IMT in the total study population [beta = 15.0 (–6.6; 36.7)], adjusted for age, gender and glucose tolerance status).

Table 1 Characteristics of the study population stratified for glucose tolerance status and gender (n = 566)

	Men			Women		
	NGT	IFG/IGT	Type 2 diabetes	NGT	IFG/IGT	Type 2 diabetes
n	109	64	104	122	63	104
Age (years)	69.3 (6.3)	68.3 (6.2)	67.2 (8.4)	68.2 (6.1)	70.9 (5.7)	69.7 (7.5)
Fasting glucose (mmol/l)	5.5 (0.4)	6.1 (0.5)	7.7 (1.9)	5.4 (0.4)	6.0 (0.5)	7.6 (1.8)
Two-hour post-load glucose (mmol/l)	5.4 (1.1)	7.7 (1.8)	11.2 (2.6)	5.7 (1.1)	8.0 (1.5)	12.1 (2.9)
Triglycerides (mmol/l)	1.3 (0.9–1.5)	1.3 (1.0–1.8)	1.5 (1.1–2.0)	1.1 (0.8–1.5)	1.3 (0.9-1.8)	1.6 (1.1–2.0)
HDL cholesterol (mmol/l)	1.33 (0.37)	1.29 (0.34)	1.14 (0.30)	1.70 (0.41)	1.63 (0.45)	1.39 (0.34)
LDL cholesterol (mmol/l)	3.6 (0.9)	3.8 (0.9)	3.4 (0.8)	3.9 (0.9)	4.0 (1.0)	3.7 (0.9)
LDL size (nm)	21.51 (0.34)	21.44 (0.38)	21.20 (0.44)	21.80 (0.32)	21.58 (0.44)	21.44 (0.48)
Total cholesterol (mmol/l)	5.5 (0.9)	5.7 (1.0)	5.3 (0.9)	6.2 (0.9)	6.3 (1.0)	6.0 (1.0)
BMI (kg/m^2)	25.8 (3.1)	26.7 (2.9)	28.6 (3.4)	26.0 (3.0)	27.9 (4.6)	28.5 (4.1)
Waist (cm)	95.2 (9.5)	98.8 (9.4)	103.5 (10.3)	85.7 (9.3)	93.1 (10.9)	95.5 (10.9)
Systolic blood pressure (mmHg)	140 (18)	140 (16)	145 (20)	137 (22)	144 (19)	147 (21)
Diastolic blood pressure (mmHg)	83 (10)	84 (11)	85 (10)	81 (12)	81 (10)	84 (10)
Current smoker (%)	18.3	21.9	13.5	13.1	11.1	11.7
Alcohol intake (g/day)	11.1 (2.4–27.4)	13.3 (4.1–33.1)	9.4 (2.6–26.9)	4.4 (0.7–12.1)	2.9 (0.4–11.8)	0.4 (0–5.9)
CETP (mg/l)	1.75 (0.52)	1.88 (0.61)	1.70 (0.59)	2.07 (0.58)	2.14 (0.56)	2.02 (0.68)
–629 CC/CA/AA (%)	34/43/22	26/40/33	26/47/27	28/51/21	27/41/32	28/51/21
Carotid IMT (μm)	854 (180)	877 (183)	880 (196)	798 (144)	854 (132)	885 (156)
Cardiovascular disease (%)*	44.9	47.6	52.6	41.8	45.9	61.0

*Cardiovascular disease defined as ankle-brachial index < 0.90, ECG abnormalities, history of arterial surgery, cerebral vascular event, angina, amputation, claudication or possible myocardial infarction.

Table 2 Associations [OR (95% CI)] of CETP with CVD stratified for glucose tolerance status and gender

	Men			Women		
	NGT	IFG/IGT	Type 2 diabetes	NGT	IFG/IGT	Type 2 diabetes
Age-adjusted*	1.22 (0.54;2.78)	1.51 (0.60;3.80)	1.26 (0.62;2.57)	0.66 (0.35;1.26)	1.24 (0.49;3.16)	3.34 (1.56;7.14)
Age and HDL-c adjusted	1.22 (0.53;2.78)	1.61 (0.63;4.12)	1.55 (0.70;3.45)	0.67 (0.35;1.29)	1.21 (0.46;3.20)	3.37 (1.57;7.24)
Age and triglycerides adjusted	1.18 (0.51;2.72)	1.51 (0.59;3.88)	1.10 (0.51;2.39)	0.66 (0.34;1.26)	1.20 (0.47;3.07)	3.33 (1.54;7.22)
Multiple adjusted†	1.10 (0.44;2.74)	1.21 (0.44;3.34)	1.58 (0.69;3.62)	0.50 (0.25;1.01)	1.41 (0.53;3.72)	3.25 (1.44;7.35)

*OR per 1 mg/l increase in CETP concentration.
†Adjusted for age, BMI, total cholesterol, systolic blood pressure and smoking.

No statistical significant interactions were found between CETP concentration and smoking, triglycerides or study population with regard to either prevalence of CVD or IMT.

629C/A polymorphism

Frequencies of the –629C/A polymorphism were equally distributed over categories of glucose tolerance status. The number of A alleles of the –629C/A polymorphism was associated with lower CETP concentration (P < 0.01) and with higher HDL-c levels (P = 0.01). The –629C/A polymorphism was not associated with CVD or IMT (data not shown).

Discussion

This population-based study showed a strong and independent association between fasting CETP concentration and the prevalence of CVD in women with Type 2 diabetes, while no clear associations with IMT were observed.

This is in line with the hypothesis that CETP should be more detrimental in patients with diabetes than in subjects with NGT [23]. Subjects with diabetes or IGT have, in addition to glucose abnormalities, elevated concentrations of triglycerides and cholesterol and decreased HDL-c levels [24]. In patients with high triglyceride levels, CETP preferentially directs CE from HDL to larger VLDL [25]. In this CETP-mediated

Table 3 Associations [β (95% CI)] of CETP with carotid IMT stratified for glucose tolerance status and gender

	Men			Women		
	NGT	IFG/IGT	Type 2 diabetes	NGT	IFG/IGT	Type 2 diabetes
Age adjusted*	2.3 (–59.7;64.3)	27.6 (–41.4;96.7)	4.0 (–55.7;63.7)	40.0 (–0.6;80.5)	21.5 (–30.6;73.6)	–10.1 (–54.4;34.2)
Age and HDL-c adjusted	1.9 (–60.4;64.2)	31.1 (–38.1;100.2)	7.6 (–52.4;67.7)	39.8 (–1.1;80.7)	14.8 (–40.0;69.6)	–12.2 (–56.8;32.3)
Age and triglycerides adjusted	6.1 (–56.2;68.4)	39.3 (–30.7;109.2)	0 (–61.4;61.3)	39.0 (–1.7;79.7)	17.5 (–34.2;69.2)	–9.5 (–54.0;35.1)
Multiple adjusted†	–7.6 (–73.5;58.3)	16.9 (–56.2;89.9)	13.5 (–52.8;79.9)	39.4 (–2.2;81.0)	18.0 (–35.3;71.3)	–30.2 (–77.5;17.1)

*Beta in µm IMT per 1 mg/l increase in CETP concentration.
†Adjusted for age, BMI, total cholesterol, systolic blood pressure and smoking.

transport, CE is exchanged for triglycerides, which are directed to LDL and HDL. These LDL and HDL particles become triglyceride enriched, providing a substrate for hepatic lipase to generate small dense LDL and HDL, which are considered to be atherogenic [4]. In addition, CETP contributes to the atherogenity of small dense LDL in an indirect way by enrichment of VLDL particles with CE. These large, CE-enriched VLDL particles are a precursor of small dense LDL [23]. Therefore, high levels of CETP concentration are expected to be atherogenic, particularly in subjects with a large pool of triglyceride-enriched lipoproteins, i.e. VLDL.

However, despite the association with CVD in women with Type 2 diabetes, we did not observe an association between CETP concentration and IMT. This may be related to a number of limitations of our study. Although several studies reported a high correlation between CETP concentration and CETP activity [26–28], the weak associations between CETP concentration and IMT could be the result of measuring CETP concentration instead of CETP activity. The relation between CETP activity and CETP concentration could differ in diabetic individuals compared with non-diabetic subjects [29]. Indeed, a recent study showed an association between CETP activity and IMT, while no association was found between CETP concentration and IMT [29]. Furthermore, measuring fasting CETP concentration may be less relevant than measuring postprandial CETP concentration to the risk of atherosclerosis and CVD, especially in diabetes. Postprandial changes in CETP concentration and activity were found in two studies [30,31], although not in one small study [32].

Until now, no other studies examined the association between CETP concentration and CVD in different categories of glucose tolerance. The finding that CETP concentration and CVD are not related to each other in non-diabetic subjects is in line with previous studies [6–9]. The beneficial effects of CETP in reverse cholesterol transport might be dominant in healthy subjects who have efficient hepatic uptake of cholesterol.

A gender difference regarding the association between CETP and CVD has not been observed previously. The association between CETP concentration and CVD in women with Type 2 diabetes was, however, very strong and of similar magnitude in women from the Hoorn Screening Study and in women from the original Hoorn Study cohort. It has been previously shown that, in women, the relative risk of CVD conferred by diabetes is greater than in men [33]. In view of the gender difference in the association between CETP and CVD in the current study, CETP might be a mediator of CVD risk. In line with previous findings, the women studied here had significantly higher levels of CETP than men, which might reflect a higher neutral lipid exchange rate between lipoproteins. In the presence of a high concentration of VLDL particles, as in diabetes [34], a high CETP concentration may contribute to the high risk of CVD in diabetic women [35]. An additional possible explanation of the lack of association in men may be selective survival. In this study population, women with diabetes were older than men (men aged 67.2 years and women

aged 69.7 years). Thus, in men who had an atherogenic lipid profile, high CETP concentration and CVD may already have deceased, in contrast to the surviving women.

We also found that the AA genotype was associated with an anti-atherogenic lipid profile. As expected, the AA genotype of the –629 C→A polymorphism was associated with lower CETP concentration and with higher HDL-c levels [19], which is generally regarded as an anti-atherogenic lipid profile. Other studies have shown these differential effects on atherosclerosis and CVD [36,37]. However, we found no associations between the CETP gene and CVD or IMT. The lack of association in the present study may be as a result of low statistical power. Indeed, in a recent meta-analysis [38], the TaqIB polymorphism of the CETP gene (acknowledged to be a marker for the functional –629C/A polymorphism) was associated with cardiovascular events, whereas no association was found in most of the individual studies included in the meta-analysis.

The current cross-sectional study design limits interpretation of our results. Patients who have had diabetes for several years receive blood glucose-lowering therapy and often change their lifestyle. As a consequence of these interventions, regression of IMT [39] and changes in CETP concentration may occur, while history of CVD remains unchanged. This could explain the discrepancies observed in the association of CETP with IMT and with CVD in the diabetic patients. In addition, exclusion of subjects who used lipid-lowering medication possibly resulted in a study population with a relatively low cardiovascular risk profile. This may have led to an underestimation of the true association between CETP concentration and CVD and/or IMT. Because of limited statistical power, we used a broad definition of CVD compared with other studies [6]. Further study into more specific CETP-related cardiovascular abnormalities might be helpful to elucidate a possible mechanistic pathway between CETP and CVD. Furthermore, the current data warrant further investigation into the value of CETP concentration in larger prospective studies, which are unfortunately very limited thus far [6–8].

In summary, associations of CETP concentration with atherosclerosis and CVD were dependent on glucose tolerance status and gender. The finding that high CETP concentration was strongly associated with prevalent CVD in women with Type 2 diabetes suggests that further investigation into CETP as a possible mediator for CVD risk in patients with diabetes is required.

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Competing interests

None declared.

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