

Plasma levels of lecithin:cholesterol acyltransferase and risk of future coronary artery disease in apparently healthy men and women: a prospective case-control analysis nested in the EPIC-Norfolk population study

A. G. Holleboom,^{1,*} J. A. Kuivenhoven,^{1,2,*} M. Vergeer,^{*} G. K. Hovingh,^{*} J. N. van Miert,^{*} N. J. Wareham,[†] J. J. P. Kastelein,^{*} K-T. Khaw,[§] and S. M. Boekholdt^{*}

Department of Vascular Medicine,^{*} Academic Medical Center, Amsterdam, The Netherlands; and Medical Research Council Epidemiology Unit[†] and Institute of Public Health,[§] University of Cambridge, Cambridge, UK

Abstract LCAT plays a key role in the maturation of HDL, as evidenced by low HDL-cholesterol levels in carriers of deleterious mutations in LCAT. However, the role of LCAT in atherosclerosis is unclear. We set out to study this in a prospective study. Plasma LCAT levels, which strongly correlate with LCAT activity, were measured in baseline non-fasting samples of 933 apparently healthy men and women who developed coronary artery disease (CAD) and 1,852 matched controls who remained free of CAD during 6 year follow-up. LCAT levels did not differ between cases and controls but were higher in women than men. Stratification into LCAT quartiles revealed a positive association with plasma LDL-cholesterol and triglyceride levels in the unexpected absence of an association with HDL-cholesterol. In mixed-gender analyses, the odds ratio (OR) for future CAD in the highest LCAT quartile versus the lowest was 1.00 [confidence interval (CI): 0.76–1.29, *P* for linearity = 0.902], although opposite trends were observed in men and women. In fact, high LCAT levels were associated with an increased CAD risk in women (unadjusted OR 1.45, CI: 0.94–2.22, *P* for linearity = 0.036). In contrast to our studies in carriers of LCAT mutations, the current data show that low LCAT plasma levels are not associated with increased atherosclerosis in the general population.—Holleboom, A. G., J. A. Kuivenhoven, M. Vergeer, G. K. Hovingh, J. N. van Miert, N. J. Wareham, J. J. P. Kastelein, K-T. Khaw, and S. M. Boekholdt. Plasma levels of lecithin:cholesterol acyltransferase and risk of future coronary artery disease in apparently healthy men and women: a prospective case-control analysis nested in the EPIC-Norfolk population study. *J. Lipid Res.* 2010. 51: 416–421.

Supplementary key words high-density lipoprotein cholesterol • atherosclerosis • lipoproteins • epidemiology • European Prospective Investigation into Cancer and Nutrition

LCAT hydrolyzes the sn-2 acyl group of phosphatidylcholine and subsequently transfers and esterifies the fatty acid to free cholesterol, thereby using apolipoprotein (apo) A-I as cofactor (1). The reaction products are thus cholesteryl ester (CE) and lysophosphatidylcholine. The vast majority of CE in the blood circulation is generated by this enzymatic reaction. LCAT is primarily active on HDL and as such drives the maturation of small nascent HDL discs to larger spherical HDL (2). In catalyzing the esterification of free cholesterol, LCAT has been proposed to maintain a concentration gradient of free cholesterol from cells to HDL, thereby facilitating reverse cholesterol transport (3, 4). LCAT-deficient patients present with almost complete HDL deficiency because they are unable to form mature HDL, which in turn leads to a rapid clearance of nascent HDL from the circulation (2, 5).

Based on our current understanding of HDL metabolism, it is not clear whether LCAT is pro- or anti-atherogenic (6, 7): the generation of CE on HDL by LCAT can be regarded as anti-atherogenic since this action increases HDL cholesterol (HDL-C) levels, but in the presence of cholesteryl ester transfer protein (CETP) and triglyceride-rich lipoproteins, the CE will be transferred to apoB-

Abbreviations: apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL-C, high density lipoprotein-cholesterol; LCL-C, low density lipoprotein-cholesterol; OR, odds ratio; SNP, single nucleotide polymorphism.

¹A. G. Holleboom and J. A. Kuivenhoven contributed equally to this work.

²To whom correspondence should be addressed.
e-mail: j.a.kuivenhoven@amc.uva.nl

HDL research by the group of Dr. Jan Albert Kuivenhoven is supported by a grant of the European Community (FP6-2005-LIFESCIHEALTH-6; STREP contract number 037631). A.G. Holleboom is supported by a grant of the Netherlands Organisation for Scientific Research (NWO; project number 021.001.035).

Manuscript received 6 April 2009 and in revised form 21 July 2009.

Published, JLR Papers in Press, August 11, 2009
DOI 10.1194/jlr.P900038-JLR200

containing lipoproteins that are atherogenic. Animal studies have, unfortunately, not provided clear answers: both LCAT knockout mice and LCAT overexpression models yielded mixed results with respect to atherogenesis, as recently reviewed by Ng (8). Recently, Amar, Shamburek, and Vaisman (9) studied adenoviral expression of human LCAT in squirrel monkeys, which express CETP at a level comparable to humans and develop diet-induced atherosclerosis. In this study, overexpression of LCAT led to an antiatherogenic lipoprotein phenotype by increasing HDL-C and lowering LDL-cholesterol (LDL-C).

Contrary to the numerous animal studies on LCAT and atherosclerosis, only very few studies were carried out in humans, but these have also provided conflicting results (5, 10–15). Genetic association studies have, historically, not been performed due to the lack of frequent LCAT gene variation in the general population. Recently, however, genome-wide association studies reported two single nucleotide polymorphisms (SNPs) near the LCAT gene locus that were associated with HDL-C levels (16, 17). Willer, Sanna, and Jackson (17) showed that the rs255052 SNP, located 49 kb downstream of LCAT, had an effect size on HDL-C of +0.019 mmol/l, while this SNP was not found associated with coronary artery disease (CAD). Another recent article described a SNP 7.7 kb upstream of LCAT to be correlated with HDL-C, but this effect was not reproduced in another cohort (18). To date, it remains to be shown whether these SNPs are indeed associated with transcriptional changes at the LCAT gene locus.

Large LCAT studies using biochemical means have been difficult to conduct because LCAT activity measurements are cumbersome and time-consuming (19). In this light, only two reports on small cross-sectional studies have shown that LCAT activity is low in patients with either angiographically documented CAD (12) or acute myocardial infarction (13). Finally, the use of LCAT plasma levels as a tool was until recently very limited due to the absence of commercially available LCAT antibodies. Families with LCAT deficiency syndromes have thus far been one of the few sources to study LCAT and atherosclerosis in man. We have previously shown that loss of LCAT function in carriers of LCAT gene mutations is associated with an increased carotid intima media thickness, a surrogate marker for cardiovascular endpoints (15). To shed more light on the role of LCAT in human atherosclerosis, the objective of this study was to assess the association between plasma LCAT levels and risk of future CAD events in a large cohort representing the general population.

MATERIALS AND METHODS

Study design and participants

A prospective nested case-control study was performed among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study. The EPIC-Norfolk co-

hort has been described in detail (20). For this analysis, only individuals who did not report a history of heart attack or stroke at the baseline clinic visit were enrolled, while participants treated with lipid-lowering medication at baseline were excluded. Cases were 993 individuals who had a hospital admission and/or died from CAD as underlying cause during follow-up (average of 6 years). CAD was defined as code 410 to 414 according to International Classification of Diseases-9th revision. Controls ($n = 1,852$) were individuals who remained free of CAD during follow-up. Two controls were matched to each case for sex, age (within 5 years), general practice, and time of enrolment (within 3 months). The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave signed informed consent.

Biochemical analysis

Nonfasting blood samples were drawn into plain and citrate bottles. Blood samples were processed directly 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 samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK). LDL-C levels were calculated using the Friedewald formula. Serum levels of apoA-I and apoB were measured by rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards (21). Serum concentrations of apoA-II were measured with a commercially available immunoturbidimetric assay (Wako Pure Chemicals Industries, Osaka, Japan) on a Cobas-Mira autoanalyzer (Roche, Basel, Switzerland). Lipoprotein subclass concentrations and average size of LDL and HDL were measured by NMR spectroscopic assay (LipoScience, NC) (22). Gradient gel electrophoresis was performed as described (23, 24). CETP concentrations were measured with a validated two-antibody sandwich-type ELISA (25). LPL levels were measured in serum using a commercially available ELISA (Dainippon). LCAT plasma levels were measured using a commercially available sandwich-type ELISA (Daiichi, Japan), which has been described in detail (26). Depending on the absolute LCAT concentration, these investigators reported intra-assay variations of 2.7–5.2% with inter-assay coefficients of variation varying from 4.5–6.1%. All EPIC-Norfolk assays were carried out in random order to avoid systemic bias and were analyzed in a blinded fashion.

Statistical analysis

Baseline characteristics were compared between cases and controls using ANOVA for continuous variables and the χ^2 test for categorical variables. Plasma LCAT levels were analyzed as categorical variables after division into quartiles based on the distribution of LCAT levels in controls. Risk factor levels per quartile of LCAT plasma level were calculated. Associations between LCAT level quartiles and traditional risk factors were calculated using ANOVA for continuous variables and the χ^2 test for trend for categorical variables. In addition, Pearson correlation coefficients were calculated to assess the correlation between LCAT levels and other continuous risk factors. Conditional logistic regression analysis was used to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) as an estimate of the relative risk of incident CAD, taking into account the matching for sex, age, and enrolment time. The lowest quartile was used as reference category. The ORs were adjusted for the cardiovascular risk factors included in the Framingham risk score: systolic blood pressure, LDL-C, HDL-C, smoking, diabetes mellitus, and CETP. Statistical analyses were performed using SPSS software (version 12.0.2; SPSS Inc., Chicago, IL). A P value of <0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics of study participants

A total of 268 out of 933 cases (28.7%) died of CAD, whereas the remaining cases suffered from nonfatal CAD events. **Table 1** gives demographic and lifestyle parameters, lipids, (apo) lipoproteins, and LCAT levels of cases and controls (for sex-specific tables, see data supplementary data). Cases had a higher body mass index (BMI), were more likely to smoke, to have diabetes, and consumed less alcohol compared with controls. As expected, levels of total cholesterol, LDL-C, triglycerides, apoB, and systolic and diastolic blood pressure were significantly higher in cases than in controls, while HDL-C and apoA-I levels were significantly lower in cases.

Validation of the LCAT concentration assay

In our hands, intra-assay and inter-assay coefficients of variations for this ELISA were 1.9% and 7.4%, respectively. These data were obtained after running 176 measurements of LCAT concentration in the same pooled plasma (harvested from 160 healthy volunteers employed in our department in 2006; this pooled plasma was stored in aliquots for single use purposes).

Kobori et al. (26) previously reported strong correlations between LCAT concentration measurements with LCAT activity as assessed with a liposome substrate ($r = 0.871$, $P < 0.001$) or endogenous substrates ($r = 0.864$, $P < 0.001$). We verified this by LCAT concentration (using the same ELISA) and LCAT activity (using proteoliposomes) measurements in plasma of heterozygotes for a mutation in the LCAT gene that causes premature truncation of the mature LCAT protein, a 28 kDa protein that was intracellularly retained (27). LCAT levels were 3.44, 3.82, and 4.08 $\mu\text{g/ml}$ compared with 7.85 $\mu\text{g/ml}$ in pool plasma. Thus, LCAT levels were 44, 49, and 52% of normal in these individuals. These findings are in line with our previous

measurements using a radio-immuno assay: LCAT concentration averaged $2.5 \pm 0.4 \mu\text{g/ml}$ compared with $4.4 \pm 0.5 \mu\text{g/ml}$ in apparently healthy family controls (26). Importantly, this approximate 50% reduction of LCAT concentration is proportional to the markedly reduced LCAT activities in these heterozygotes compared with controls (19.7 ± 2.6 vs. $28.8 \pm 3.2 \text{ nmol}\cdot\text{h}^{-1}\cdot\text{ml}^{-1}$ in the controls, respectively).

Plasma LCAT levels, demographic and lifestyle parameters, and lipid parameters

Plasma LCAT levels were normally distributed and were close to identical in cases and controls [$8.89 \mu\text{g/ml}$ (SD 2.10) vs. $8.91 \mu\text{g/ml}$ (SD 2.25), respectively; $P = 0.78$]. Considering only the males, again no differences were observed [$8.69 \mu\text{g/ml}$ (SD 2.0) vs. $8.72 \mu\text{g/ml}$ (SD 2.0) among cases and controls, respectively; $P = 0.3$]. Among women, however, LCAT levels tended to be higher in cases [$9.44 \mu\text{g/ml}$ (SD 2.3) vs. $9.16 \mu\text{g/ml}$ (SD 2.3) in controls; $P = 0.06$]. When analyzing cases and controls together, average LCAT levels were significantly higher in women compared with men ($9.25 \mu\text{g/ml}$ versus $8.69 \mu\text{g/ml}$, respectively; $P < 0.001$).

Table 2 summarizes the associations of quartiles of LCAT levels with established cardiovascular risk factors and lipid profiles. LCAT levels were inversely associated with age. On the other hand, LCAT levels were positively associated with alcohol use, smoking, BMI, waist circumference, waist-to-hip ratio, and both systolic and diastolic blood pressure. The prevalence of diabetes did not differ among the quartiles. LCAT levels were positively associated with total cholesterol, LDL-C, and triglycerides. In the entire cohort, apoA-I, apoA-II, and apoB levels were all positively associated with LCAT levels ($P < 0.001$, $P < 0.0001$, and $P < 0.0001$, respectively). See supplementary data for gender-specific data. The inverse association of LCAT with age could be attributed to associations observed in only the male participants.

TABLE 1. Baseline characteristics of cases and matched controls

	Cases	Controls	P
Study population, n	933	1,852	–
Demographic and lifestyle parameters			
Age (years)	65.4 (7.7)	65.4 (7.7)	Matched
Women, % (n)	35.9 (335)	37.3 (691)	Matched
BMI (kg/m ²)	27.3 (3.9)	26.2 (3.4)	<0.001
Diabetes, % (n)	6.8 (63)	1.8 (33)	<0.001
Smokers, % (n)	15.1 (141)	8.4 (166)	<0.001
Alcohol use, units per week*	2.5 (0.5-8.0)	4.0 (1.0-10)	0.001
Systolic blood pressure (mmHg)	143.6 (18.8)	138.9 (17.9)	<0.001
Diastolic blood pressure (mmHg)	85.8 (12.1)	84.4 (11.5)	<0.001
Lipids and lipoproteins			
Total cholesterol (mmol/l)	6.45 (1.2)	6.26 (1.1)	<0.001
Triglycerides (mmol/l)*	1.9 (1.40-2.80)	1.6 (1.20-2.30)	<0.0001
LDL-C (mmol/l)	4.27 (1.1)	4.08 (1.0)	<0.001
HDL-C (mmol/l)	1.26 (0.37)	1.37 (0.40)	<0.001
apoA-I (mg/dl)	155.1 (29.5)	162.2 (28.7)	<0.01
apoB (mg/dl)	136.6 (31.7)	128.4 (29.9)	<0.001
LCAT ($\mu\text{g/ml}$)	8.89 (2.10)	8.91 (2.25)	0.78

Controls were matched to cases for age, sex, and enrolment time. Data are presented as mean values with SD in parentheses or as a percentage of the total group. P values are for one-way ANOVA on continuous variables or for χ^2 analysis on categorical variables. *Mean and interquartile range. Because of skewed distribution, data on alcohol and triglycerides were log-transformed prior to performing a one-way ANOVA test.

TABLE 2. Plasma LCAT levels, demographic and lifestyle characteristics, and lipid parameters

	LCAT Quartile				<i>P</i> ^a	<i>R</i> ^b	<i>P</i> ^b
	1 (n = 700)	2 (n = 684)	3 (n = 691)	4 (n = 710)			
Age, y	66.3 (7.4)	65.5 (7.7)	65.0 (7.9)	64.7 (7.7)	<0.001	-0.54	0.005
Current smoker, % (n)	16 (110)	10 (69)	10 (69)	6.9 (49)	<0.0001 ^c	-	-
Former smoker, % (n)	46 (321)	52 (355)	53 (366)	55 (389)	-	-	-
Never smoked, % (n)	38 (269)	38 (260)	37 (256)	38 (272)	-	-	-
Alcohol use, units/week ^d	2.5 (1.0–8.0)	3.0 (1.0–9.5)	4.0 (1.0–11)	4.0 (1.0–12)	<0.0001	0.046	0.015 ^e
BMI, kg/m ²	25.8 (3.7)	26.5 (3.4)	26.8 (3.6)	27.3 (3.6)	<0.0001	0.164	<0.0001
Waist circumference, cm	90.0 (12.2)	92.1 (11.3)	92.7 (11.0)	93.9 (11.2)	<0.0001	0.086	<0.0001
Waist hip ratio	0.88 (0.09)	0.89 (0.08)	0.89 (0.08)	0.90 (0.08)	<0.0001	0.018	0.345
Diabetes, % (n)	3.1 (22)	3.7 (25)	3.5 (24)	3.5 (25)	0.96	-	-
Systolic BP, mmHg	138 (18.5)	140 (18.9)	141 (17.4)	143 (18.1)	<0.0001	0.123	<0.0001
Diastolic BP, mmHg	82 (11.6)	84 (11.8)	85 (11.2)	86 (11.2)	<0.0001	0.138	<0.0001
Total cholesterol, mmol/l	5.9 (1.0)	6.2 (1.0)	6.4 (1.1)	6.8 (1.2)	<0.0001	0.315	<0.0001
LDL-C, mmol/l	3.8 (0.9)	4.1 (0.9)	4.2 (1.0)	4.5 (1.1)	<0.0001	0.241	<0.0001
HDL-C, mmol/l	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	0.56	0.055	0.004
Triglycerides, mmol/l ^d	1.4 (1.0–1.9)	1.6 (1.2–2.2)	1.7 (1.3–2.4)	2.0 (1.5–2.7)	<0.0001	0.356	<0.0001 ^e
apoA-I, mg/dl	155.8 (33.2)	159.4 (27.0)	161.3 (28.7)	162.7 (27.2)	<0.001	0.103	<0.0001
apoB, mg/dl	120.4 (29.3)	128.9 (29.9)	132.7 (30.5)	141.1 (30.6)	<0.0001	0.239	<0.0001

Values are mean ± SD or percentage and number of participants.

^a *P* value for ANOVA of risk factor levels between LCAT quartiles.

^b *R* indicates Pearson's correlation coefficient between LCAT levels and risk factors and the corresponding *P* value (*P*^b).

^c *P* value for smoking by χ^2 test for trend.

^d Median (interquartile range).

^e Spearman's correlation coefficient between LCAT levels and risk factors and the corresponding *P* value (*P*^b).

We did not observe a correlation between LCAT levels and HDL-C when analyzing men and women separately or when both sexes were combined. **Table 3** gives Pearson's correlations between LCAT levels and additional lipid parameters. LCAT was strongly negatively correlated with HDL size and LDL size as determined by gradient gel electrophoresis and NMR measurements. Furthermore, LCAT levels were positively associated with apoA-II, CETP, and LPL.

Plasma LCAT levels and risk of future CAD for men and women

In a mixed-gender analysis, LCAT quartiles were not associated with the risk of CAD (OR = 1.00; 95% CI 0.76–1.29 comparing the top versus bottom quartile; *P* for linearity over the quartiles 0.902; see **Table 4**). Adjustment for the Framingham risk score did not change this result (OR = 0.90; 95% CI 0.69–1.17 for a comparison of top versus bottom quartile; *P* = 0.519).

TABLE 3. Correlation of LCAT levels with lipoprotein subfractions and total numbers, apoA-II, and lipid-modifying parameters

LCAT	LCAT	
	<i>R</i>	<i>P</i>
HDL size, nm (GGE)	-0.108	<0.0001
Large HDL, nmol/l (NMR)	0.013	0.484
Medium HDL, nmol/l (NMR)	0.043	0.024
Small HDL, nmol/l (NMR)	0.403	<0.0001
LDL size, nm (GGE)	-0.180	<0.0001
Large LDL, nmol/l (NMR)	0.032	0.093
Medium small LDL, nmol/l (NMR)	0.289	<0.001
Small LDL, nmol/l (NMR)	0.294	<0.001
apoA-II, mg/l	0.302	<0.0001
CETP concentration, mg/l	0.225	<0.0001
LPL, ng/ml	-0.050	0.01

GGE indicates gradient gel electrophoresis. *R* indicates two-tailed Pearson's correlation with the corresponding *P* value.

For men, the risk of CAD decreased with increasing LCAT levels. This linearity proved statistically significant after adjustment for the Framingham risk score (OR = 0.71; 95% CI 0.51–0.97 for a comparison of top versus bottom quartile; *P* for linearity = 0.03). In contrast, the risk of CAD in women increased with increasing LCAT levels (OR = 1.35; 95% CI 0.87–2.09 for a comparison of top versus bottom quartile; *P* for linearity = 0.036). However, after adjustment for the Framingham risk score, this statistical significance was lost (*P* = 0.08; see **Table 4**). Adjustment for plasma CETP levels did not affect the results of the mixed-gender nor the gender-specific analyses.

DISCUSSION

This prospective analysis shows for the first time that low plasma levels of LCAT are not associated with an increased risk of future CAD in the general population. Although a gender-specific effect was identified that needs further investigation, this finding does not support our previous finding of increased atherosclerosis in patients with a marked loss of LCAT function (15). The current analyses furthermore show that LCAT levels were positively associated with alcohol use, smoking, BMI, waist circumference, waist-to-hip ratio, blood pressure, total cholesterol, triglycerides, and LDL-C levels in the absence of an association with HDL-C.

Plasma LCAT levels and lipid metabolism

The absence of an association between plasma LCAT levels and HDL-C was previously described by Albers et al. (28, 29) in both normolipidemic and hyperlipidemic volunteers but not in two other small cross-sectional studies in which a positive correlation between LCAT levels, LCAT activity, and HDL-C was found (30, 31). Taken the results of the current larger and prospective analysis, it could be argued

TABLE 4. Odds ratios for CAD per LCAT quartile for entire cohort and men and women separately

	LCAT Quartile				P
	1	2	3	4	
Entire cohort, n	700	684	691	710	
Unadjusted	1.00	0.87 (0.69–1.11)	0.92 (0.72–1.18)	1.00 (0.76–1.29)	0.902
Adjusted for Framingham risk score ^a	1.00	0.84 (0.66–1.08)	0.87 (0.68–1.12)	0.90 (0.69–1.17)	0.519
Men, n	453	441	434	431	
Unadjusted	1.00	0.87 (0.66–1.17)	0.82 (0.61–1.11)	0.80 (0.59–1.10)	0.2
Adjusted for Framingham risk score ^a	1.00	0.83 (0.62–1.12)	0.75 (0.55–1.02)	0.71 (0.51–0.97)	0.030
Women, n	247	243	257	279	
Unadjusted	1.00	0.88 (0.58–1.34)	1.15 (0.76–1.76)	1.45 (0.94–2.22)	0.036
Adjusted for Framingham risk score ^a	1.00	0.88 (0.57–1.35)	1.14 (0.75–1.76)	1.35 (0.87–2.09)	0.08

Odds ratios for the risk of future CAD events for both sexes and men and women separately. The corresponding CIs are in parentheses. *P* for χ^2 test for linear trend with 1 degree of freedom.

^a Framingham risk score includes age, sex, total cholesterol, HDL-C, systolic and diastolic blood pressure, smoking, and the presence of diabetes.

that LCAT concentration does not reflect LCAT activity, but there is ample evidence from other studies (26, 28, 29) that it does. We confirmed this by measuring 50% LCAT activity and 50% LCAT plasma concentration in heterozygotes for a mutation in *LCAT* that causes premature truncation of the mature LCAT protein. It can also be argued that the LCAT reaction is not a rate-limiting step in HDL genesis, but this does not agree with the notion that loss of LCAT function causes marked reduction of plasma HDL-C levels in individual with LCAT gene mutations. On the other hand, it was recently shown that SNPs near the LCAT gene locus are associated with HDL-C levels, but it remains to be shown whether these SNP are indeed associated with, e.g., transcriptional regulation of the LCAT gene (16, 17).

The positive association of LCAT levels with LDL-C levels in this study also corroborates the findings of Albers et al. (28, 29). Patients with familial LCAT deficiency present with reduced LDL-C levels (5), while kinetic analyses in humans have shown that 30% of plasma CE are formed on apoB-containing particles (32).

We also show that plasma triglycerides and waist circumference/waist-to-hip ratio are positively associated with plasma LCAT levels, which confirms an independent positive association that was recently reported between LCAT activity and presence of the metabolic syndrome (11). The respective authors postulated that increased LCAT synthesis by the liver might represent a salvage pathway against the dyslipidemia in metabolic syndrome. Whether this is true cannot be concluded from this study.

Plasma LCAT levels and risk of future CAD

This study shows that plasma LCAT levels do not predict risk of future CAD. This result is not what we expected on the basis of our previous finding of increased carotid intima media thickness in carriers of functional LCAT gene mutations compared with unaffected family members (15). This discrepancy may be explained by relatively large reductions of LCAT in our families, while the variation of LCAT concentration in the studied population is by com-

parison limited. On the other hand, a recent study showed a positive association of LCAT levels with carotid atherosclerosis in patients with the metabolic syndrome as well as in control subjects (11). Other investigators previously showed that LCAT activity was reduced in 90 patients with CAD (12) and in 60 patients with acute myocardial infarction (13, 14). Although these small studies lacked a prospective design, it can be reasoned that LCAT activity might be reduced in the acute phase of a myocardial infarction, probably in parallel with the reduced HDL-C levels, but may normalize over time (33).

Gender-specific analyses furthermore showed that higher LCAT levels in women were associated with an increased risk of CAD. In the males, an opposite nonsignificant trend was observed. It is possible that the opposite trends in men and women underlie the absence of a relation between LCAT levels and CAD in the mixed-gender analysis. At this point, we have no explanation for this effect (most women were postmenopausal at enrolment), and it should be confirmed in other sex-specific prospective studies.

Considerations

Certain aspects of this study merit further consideration. Plasma levels of LCAT were determined in a nonfasting sample; however, most studies report no large effect of diet or feeding condition on LCAT (19). Furthermore, a single measurement of LCAT plasma level may fail to decipher the role of this enzyme in the physiology of pro- and anti-atherogenic lipid changes and associated atherosclerosis. Also, CAD events were scored through death certification and hospital admission data, which may have resulted in underascertainment or misclassification. Previous validation studies in this study cohort, however, indicate high specificity of such case ascertainment (34). Finally, we adjusted for confounding factors, but residual confounding by imperfectly measured or unmeasured confounders cannot be excluded. However, this is a common limitation of a nonrandomized study. In this respect, treatment with rosuvastatin has recently been found to de-

crease LCAT activity (35). Although subjects treated with lipid-lowering therapy were excluded from this analysis, the potential initiation of such therapy during follow-up might be a confounder of unknown importance.

CONCLUSIONS

This study shows that low plasma LCAT levels (reflecting low LCAT activity) are not associated with an increased risk of future CAD in the general population. Although this may be related to opposite trends in both genders that merit further investigation, this finding does not match our finding of increased in our studies in families with LCAT gene mutations (15). ■■

We thank the participants, general practitioners, and staff of EPIC-Norfolk.

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