Plasma lecithin:cholesterol acyltransferase and carotid intima-media thickness in European individuals at high cardiovascular risk

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Abstract Lecithin:cholesterol acyltransferase (LCAT) is the enzyme responsible for cholesterol esterification in plasma. LCAT is a major factor in HDL remodeling and metabolism, and it has long been believed to play a critical role in macrophage reverse cholesterol transport (RCT). The effect of LCAT on human atherogenesis is still controversial. In the present study, the plasma LCAT concentration was measured in all subjects (n = 540) not on drug treatment at the time of enrollment in the multicenter, longitudinal, observational IMPROVE study. Mean and maximum intima-media thickness (IMT) of the whole carotid tree was measured by B-mode ultrasonography in all subjects. In the entire cohort, LCAT quartiles were not associated with carotid mean and maximum IMT (P for trend 0.95 and 0.18, respectively), also after adjustment for age, gender, HDL-cholesterol (HDL-C), and triglycerides. No association between carotid IMT and LCAT quartiles was observed in men (P=0.30 and P=0.99 for mean and maximum IMT, respectively), whereas carotid IMT increased with LCAT quartiles in women (P for trend 0.14 and 0.019 for mean and maximum IMT, respectively). The present findings support the concept that LCAT is not required for an efficient reverse cholesterol transport and that a low plasma LCAT concentration and activity is not associated with increased atherosclerosis.—Calabresi, L., D. Baldassarre, S. Simonelli, M. Gomaraschi, M. Amato, S. Castelnuovo, B. Frigerio, A. Ravani, D. Sansaro, J. Kauhanen, R. Rauramaa, U. de Faire, A. Hamsten, A. J. Smit, E. Mannarino, S. E. Humphries, P. Giral, F. Veglia, C. R. Sirtori, G. Franceschini, and E. Tremoli. Plasma lecithin:cholesterol acyltransferase and carotid intima-media thickness in European individuals at high cardiovascular risk. *J. Lipid Res.* 2011. **52:** 1569–1574.

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The lecithin:cholesterol acyltransferase (LCAT) enzyme catalyzes the transacylation of the sn-2 fatty acid of lecithin to the free 3-OH group of cholesterol, generating cholesteryl ester (CE) and lysolecithin (1). In this way, the LCAT

reaction accounts for the synthesis of most of the plasma CE. The human *LCAT* gene (4.5 kb) is localized on chromosome 16 (region 16q22) and encodes a glycoprotein of 416 residues, with a molecular weight of 63 kDa. In blood, LCAT preferentially binds to high density lipoproteins (HDL), where it is activated by apolipoprotein A-I; a minority of plasma LCAT circulates bound to apoB-containing lipoproteins (1).

LCAT has a key place in HDL metabolism, and it has long been believed to play a critical role in macrophage reverse cholesterol transport (RCT) by maintaining the cholesterol gradient between the cell membrane and extracellular acceptors (2). However, recent findings have questioned this concept. Human LCAT overexpression in mice remarkably increases plasma HDL levels but does not enhance macrophage RCT in vivo (3). Conversely, LCAT-deficient mice display a preserved macrophage RCT in vivo despite the severe plasma HDL reduction (3). These findings are consistent with data in humans, showing that sera from genetic carriers of LCAT deficiency can efficiently remove cholesterol from macrophages (4) and that HDL is capable of directly delivering large amounts of unesterified cholesterol to the liver via the scavenger receptor type BI (SR-BI) without LCAT-mediated conversion into CE (5, 6).

The effect of LCAT on atherogenesis is controversial. Investigations in various animal models overexpressing or lacking LCAT provided inconsistent results (recently reviewed in Ref. 7). LCAT overexpression in mice or rabbits remarkably increased plasma HDL levels and was associated with protection against diet-induced atherosclerosis in rabbits but not in mice. Mice lacking the *LCAT* gene showed the expected reductions in plasma HDL levels but had less or more atherosclerosis than their counterparts with active LCAT, mostly depending on the plasma levels of apoB-containing lipoproteins.

The relationship between LCAT and vascular disease in humans has been investigated in a limited number of studies, also with conflicting results. Early cross-sectional investigations reported either decreased (8, 9) or increased (10) LCAT activity in patients with angiographically proven coronary artery disease (CAD). More recently, higher plasma LCAT activity was associated with increased incidence of CAD in men participating in the population-based PREVEND study (11). However, no association was found between plasma LCAT concentration, which strongly correlates with LCAT activity, and future cardiovascular events in a larger cohort of men from the EPIC-Norfolk population study (12). Notably, in this latter study, higher plasma LCAT levels were instead associated with a greater risk of CAD in women (12). Carotid intima-media thickness (IMT) is one of the best established and most commonly used measures of preclinical atherosclerosis (13). It correlates with many cardiovascular risk factors, including low plasma HDL-C levels (14, 15), and with the severity of CAD (16). Most importantly, in prospective studies, carotid IMT predicts clinical CAD (17). In the present study, we measured plasma LCAT concentration in European men and women at high cardiovascular risk

to explore the relationship between LCAT and preclinical atherosclerosis, as assessed by carotid IMT.

MATERIALS AND METHODS

Participants

A total of 540 individuals free of any pharmacological treatments were enrolled among those recruited in the IMPROVE study, a multicenter, longitudinal, observational study, funded by the European Union within the Fifth Framework Programme. The IMPROVE study (18) started in March 2004, involved seven centers in five European countries (Finland, France, Italy, the Netherlands, and Sweden), and recruited a total of 3,711 individuals (1,050 in Finland, 501 in France, 1,095 in Italy, 532 in the Netherlands, and 533 in Sweden) who met the eligibility criteria. The criteria were as follows: 54- to 79-year-old men and women who had at least three cardiovascular risk factors (dyslipidemia, hypertension, diabetes, smoking, or family history of cardiovascular disease), who were asymptomatic for cardiovascular disease, and who were free of any conditions that might limit longevity or carotid IMT visualization (18). All individuals not on drug therapy at the time of recruitment were included in the present analysis. All patients gave written, informed consent.

Biochemical analyses

Blood was collected after an overnight fast. Plasma samples were kept at –80°C prior to shipment to Milano and were not thawed before analysis. Plasma total and unesterified cholesterol, HDL-C, and triglyceride levels were determined with certified enzymatic methods by using a Roche diagnostics Integra 400 autoanalyzer. Plasma LDL-cholesterol was calculated with the Friedewald's formula. Plasma LCAT concentration was measured by an immunoenzymatic assay developed in our laboratory (19). Briefly, this is a competitive ELISA using an anti-peptide rabbit antibody generated against the peptide corresponding to residues 393-416 of human LCAT as primary antibody and using peroxidase-conjugated goat anti-rabbit immunoglobulins as secondary antibody. The intra-assay and inter-assay CVs are 3% and 9%, respectively.

Ultrasonographic variables

Carotid ultrasonography was performed as described (18). Briefly, the far walls of the left and right common carotids, bifurcations, and internal carotids were visualized in anterior, lateral, and posterior projections and recorded on sVHS videotapes. Carotid IMT measurements were performed in a centralized laboratory (Department of Pharmacological Sciences, University of Milan, Italy) using a dedicated software (M'Ath, Metris S.R.L., France). All carotid measurements were averaged to calculate the IMT_{mean} for each subject; the highest IMT value among the three carotid segments was defined as the IMT_{max}. Repeatability of carotid IMT measurements was previously described (18).

Statistical analyses

Numerical variables were summarized as means and 95% confidence intervals (CI). Data with skewed distributions (triglycerides and carotid IMT measurements) were summarized as geometric means and 95% CI and log-transformed before further analyses. Categorical variables were summarized as percentages. Clinical and anthropometric variables were compared between genders by unpaired Students' *t*-test or Chi-square test, as appropriate. Linear associations between LCAT and carotid IMT measurements, crude or adjusted for potential confounders, were

tested by ANCOVA. All tests were two-sided, and *P* values below 0.05 were regarded as significant. In gender-specific analysis of the relationship between LCAT and IMT, the significance threshold was set at 0.025 to account for multiple comparisons. The sample size of 540 subjects yielded 80% statistical power to detect as significant a correlation coefficient greater than 0.12 or greater than 0.17 in the analysis stratified by gender. All analyses were performed using the SAS statistical package (SAS Institute Inc., Cary, NC) version 9.13.

RESULTS

Baseline characteristics of study participants

A total of 540 subjects, 247 women and 293 men, were enrolled in the study. Almost half of the participants were hypertensive, 56% of them were current or former smokers, and 11% had diabetes (**Table 1**). On average, plasma total and LDL-cholesterol levels were slightly above the upper normal limit, while HDL-C and triglyceride levels were within the normal range (Table 1). Plasma LCAT concentration was also within the normal range (3.1-6.7 μg/ml).

The prevalence of current/former smokers and diabetes was lower in women than men (Table 1). Women had significantly higher total, LDL-, and HDL-cholesterol levels than men, but they had lower triglycerides. The average plasma LCAT concentration was similar in women and men (Table 1).

Plasma LCAT concentration and lipid levels

In the whole group of examined subjects, plasma LCAT concentration correlated positively with HDL-C ($R = 0.146$, $P = 0.0007$) and negatively with triglycerides ($R = -0.086$, $P = 0.046$). As expected, plasma LCAT concentration also correlated significantly and negatively with the unesterified/total cholesterol ratio ($R = -0.140$, $P = 0.002$), confirming that, in the absence of genetic defects, LCAT concentration reflects enzyme activity.

Plasma LCAT concentration and carotid IMT

Table 2 shows the characteristics of participants stratified by quartiles of plasma LCAT concentration. In the whole group of subjects, plasma LCAT concentration was not associated with carotid IMT_{mean} and IMT_{max} (**Table 3**). Adjustment for age, gender, HDL-C, and triglycerides did not change the results. No association between LCAT concentration and carotid IMT was observed in men, either before or after adjustment for age, HDL-C, and triglycerides (Table 3). In contrast, carotid IMT increased with LCAT quartiles in women (Table 3). The association between LCAT and IMT_{max} was positive and significant, although losing full significance after adjustment for age, HDL-C, and triglycerides. Nevertheless, the interaction gender × LCAT was not significant ($P = 0.19$). The association between LCAT and IMT_{mean} was also positive but not significant.

DISCUSSION

The present study shows that low plasma levels of LCAT are not associated with increased carotid IMT in individuals at high cardiovascular risk. However, gender-specific analysis shows that low plasma LCAT levels are associated, although with borderline significance, with decreased carotid IMT in women. These results are in agreement with a recent study showing that low LCAT activity is associated with reduced carotid IMT in a small group of patients with the metabolic syndrome (20). Carotid IMT, one of the best established and most commonly used surrogate markers of human atherosclerosis (13), correlates with the severity of coronary heart disease (CHD) (16) and predicts clinical CHD in prospective studies (17). We have not analyzed cardiovascular events in the studied population, but the relation we have observed between LCAT and carotid IMT likely reflects a similar relation between LCAT and events. Indeed, recent findings have shown that low plasma LCAT levels are not associated with increased vascular

TABLE 1. Characteristics of participants

	Entire Cohort	Women	Men	P^a
Study population (n)	540	247	293	
Age (y)	63.7 (63.3, 64.2)	63.7 (63.1, 64.3)	63.7 (63.1, 64.3)	0.91
Male gender (%)	54%	0%	100%	
Smoking status (%)				< 0.0001
Current	21%	14%	28%	
Former	35%	27%	41%	
Never	44%	58%	31%	
BMI (kg/m^2)	26.3 (26.0, 26.6)	26.1 (25.5, 26.7)	26.5 (26.2, 26.9)	0.20
Diabetes (%)	11%	7%	15%	0.004
Hypertensive (%)	49%	46%	51%	0.24
Total cholesterol (mg/dl)	233.6 (230.4, 236.9)	243.5 (238.5, 248.4)	225.3 (221.3, 229.4)	< 0.0001
Unesterified cholesterol (mg/dl)	65.5 (64.5, 66.4)	68.6 (67.3, 70.0)	62.8 (61.6, 64.1)	< 0.0001
LDL cholesterol (mg/dl)	158.4 (155.5, 161.3)	163.9 (159.5, 168.4)	153.7 (150.0, 157.3)	0.0005
HDL cholesterol (mg/dl)	50.2 (48.9, 51.4)	56.0 (54.0, 58.0)	45.2 (43.8, 46.6)	< 0.0001
Triglycerides^b (mg/dl)	112.2 (107.8, 117.9)	103.5 (97.5, 109.9)	120.3 (113.3, 129)	0.0007
LCAT (μg/ml)	5.02 (4.90, 5.13)	5.06 (4.89, 5.24)	4.98 (4.83, 5.13)	0.48

Results are expressed as means (95% CI) or percentages.

BMI, body mass index.

^{a}Women versus men compared by Student's *t*-test or Chi-square test for numerical and categorical variables, respectively.

^{b}Geometric means (95% CI). Data were log-transformed before analysis.

TABLE 2. Demographic characteristics and plasma lipid levels per LCAT quartiles

	LCAT Quartiles				
	1st (n = 135)	2nd (n = 133)	3rd (n = 137)	4th (n = 135)	P_{Trend}
LCAT (µg/ml) (mean, range)	3.35 (1.76-4.08)	4.54 (4.09-4.99)	5.42 (5.01-5.85)	6.75 (5.86-10.30)	
Age (y)	63.4 (62.5, 64.3)	63.2 (62.4, 64.1)	63.9 (63.1, 64.7)	64.2 (63.3, 65.0)	0.07
Male gender (%)	58%	55%	53%	51%	0.26
Smoking status (%)					0.78
Current	21%	20%	23%	21%	
Former	38%	34%	34%	35%	
Never	41%	46%	43%	44%	
BMI (kg/m^2)	26.6 (26.0, 27.3)	26.2 (25.6, 26.8)	26.0 (25.3, 26.6)	26.5 (25.8, 27.2)	0.41
Diabetes (%)	13%	10%	10%	11%	0.61
Hypertensive (%)	39%	52%	53%	50%	0.08
Total cholesterol (mg/dl)	231.3 (224.5, 238.1)	229.9 (223.0, 236.9)	235.2 (228.7, 241.8)	237.9 (232.3, 243.6)	0.06
Unesterified cholesterol (mg/dl)	67.0 (65.2, 68.8)	64.7 (62.7, 66.7)	64.5 (62.7, 66.4)	65.6 (63.7, 67.5)	0.42
Unesterified/total cholesterol ratio	0.292 (0.285, 0.3)	0.286 (0.278, 0.293)	0.278 (0.271, 0.285)	0.278 (0.271, 0.284)	0.0009
LDL cholesterol (mg/dl)	155.5 (149.3, 161.9)	155.5 (149.5, 161.4)	161.3 (155.3, 167.3)	161.1 (156.2, 166.0)	0.05
HDL cholesterol (mg/dl)	48.1 (45.6, 50.7)	48.0 (45.8, 50.3)	51.8 (49.5, 54.1)	52.6 (49.7, 55.5)	0.002
Triglycerides[a] (mg/dl)	125.2 (114.4, 137.0)	114.4 (103.5, 126.5)	102.5 (94.6, 111.1)	109.9 (101.5, 120.3)	0.07

Results are expressed as means (95% CI) or percentages. P values for trends were computed by linear regression for continuous variables.
BMI, body mass index.
[a] Geometric means (95% CI).

events in the general population (11, 12) and that low plasma LCAT levels are associated with decreased vascular events in women (12).

The present results are fully consistent with those of our recent study of individuals with genetic LCAT deficiency (21), where carotid IMT was measured in 40 carriers of *LCAT* gene mutations (12 carriers of two mutant *LCAT* alleles and 28 heterozygotes) from Italian LCAT-deficient families (22) and then compared with 80 matched healthy controls. Carriers of two mutant *LCAT* alleles had extremely reduced HDL-C levels and heterozygotes had lower HDL-C levels than matched controls. However, the mean and maximum IMT values in carriers were 0.07 mm and 0.21 mm smaller than in controls; moreover, the inheritance of a mutated LCAT genotype had a remarkable gene-dose-dependent effect in reducing carotid IMT (21).

The observation that reduced plasma LCAT concentrations leading to reduced HDL-C levels do not result in increased atherosclerosis is quite surprising because of the critical role that LCAT has been believed to play in RCT. LCAT has long been believed to be necessary for macrophage cholesterol efflux and RCT by maintaining the free cholesterol gradient between cell membranes and plasma acceptors (2). However, recent evidence has questioned this concept. First, sera from humans and mice lacking LCAT activity can efficiently remove cholesterol from macrophages via the ABCA1 transporter (3, 4), likely the major player in cholesterol removal from macrophages (23, 24), in a process less sensitive to cholesterol gradient than passive diffusion. Second, LCAT-deficient mice display a preserved macrophage-to-feces RCT in vivo (3), in agreement with the observation that unesterified cholesterol within HDL may be efficiently delivered to the liver and excreted into the bile without conversion into CE (5, 6). Thus, although LCAT is clearly important for HDL remodeling and metabolism, it may not be as critical in maintaining a normal rate of macrophage RCT.

Limitations

The studied population only included high-risk patients, thus limiting the extrapolation of the present findings to the general population. Nevertheless, the major findings of the study are fully consistent with those obtained in the EPIC-Norfolk general population (12),

TABLE 3. Carotid IMT per LCAT quartiles

	LCAT Quartiles					
	1st	2nd	3rd	4th	P_{trend}	P_{trend}[a]
Whole group (n)	135	133	137	135		
IMT$_{Mean}$ (mm)	0.94 (0.93, 0.96)	0.93 (0.92, 0.94)	0.93 (0.92, 0.95)	0.94 (0.93, 0.96)	0.95	0.90
IMT$_{Max}$ (mm)	1.28 (1.24, 1.32)	1.27 (1.23, 1.30)	1.28 (1.25, 1.31)	1.31 (1.28, 1.35)	0.18	0.17
Men (n)	78	73	73	69		
IMT$_{Mean}$ (mm)	0.98 (0.96, 1.00)	0.95 (0.93, 0.97)	0.96 (0.94, 0.97)	0.97 (0.95, 0.99)	0.30	0.32
IMT$_{Max}$ (mm)	1.35 (1.30, 1.40)	1.31 (1.26, 1.36)	1.33 (1.29, 1.37)	1.35 (1.30, 1.41)	0.99	0.96
Women (n)	57	60	64	66		
IMT$_{Mean}$ (mm)	0.89 (0.88, 0.91)	0.90 (0.89, 0.92)	0.91 (0.89, 0.93)	0.92 (0.89, 0.94)	0.14	0.24
IMT$_{Max}$ (mm)	1.19 (1.15, 1.23)	1.22 (1.17, 1.26)	1.23 (1.17, 1.28)	1.27 (1.22, 1.33)	0.019	0.05

Results are expressed as means (95% CI). P values for trends were computed by linear regression for continuous variables.
[a] Adjusted for age, (gender), HDL-C, and triglycerides.

indicating that the relationship between LCAT and atherosclerosis persists over a wide range of cardiovascular risk. Such a conclusion is strengthened by inclusion in the present study of individuals from all over Europe, thus minimizing the impact of genetic and environmental factors.

CONCLUSION

The present study, together with the recent analysis of the EPIC-Norfolk population (12) and in agreement with our study of Italian LCAT-deficient subjects (21), supports the concept that a low plasma LCAT concentration and/ or activity is not associated with increased preclinical atherosclerosis and cardiovascular risk. This is true despite the low plasma HDL-C levels of subjects with low LCAT concentration, as shown here and in carriers of genetic LCAT deficiency (21). Furthermore, gender-specific analysis in the present study and of the EPIC-Norfolk populations (12) indicate that, in women, low plasma LCAT concentrations are associated with decreased preclinical atherosclerosis and cardiovascular risk. Altogether, these findings challenge the long-held belief that LCAT is atheroprotective, and they support the concept that LCAT is not required for an efficient RCT (3, 4) and that a low plasma LCAT concentration and activity is not associated with increased atherosclerosis (21).

APPENDIX

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