Recombinant LCAT (Lecithin:Cholesterol Acyltransferase) Rescues Defective HDL (High-Density Lipoprotein)-Mediated Endothelial Protection in Acute Coronary Syndrome

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Objective—Aim of this study was to evaluate changes in LCAT (lecithin:cholesterol acyltransferase) concentration and activity in patients with an acute coronary syndrome, to investigate if these changes are related to the compromised capacity of HDL (high-density lipoprotein) to promote endothelial nitric oxide (NO) production, and to assess if rhLCAT (recombinant human LCAT) can rescue the defective vasoprotective HDL function.

Approach and Results—Thirty ST-segment–elevation myocardial infarction (STEMI) patients were enrolled, and plasma was collected at hospital admission, 48 and 72 hours thereafter, at hospital discharge, and at 30-day follow-up. Plasma LCAT concentration and activity were measured and related to the capacity of HDL to promote NO production in cultured endothelial cells. In vitro studies were performed in which STEMI patients' plasma was added with rhLCAT and HDL vasoprotective activity assessed by measuring NO production in endothelial cells. The plasma concentration of the LCAT enzyme significantly decreases during STEMI with a parallel significant reduction in LCAT activity. HDL isolated from STEMI patients progressively lose the capacity to promote NO production by endothelial cells, and the reduction is related to decreased LCAT concentration. In vitro incubation of STEMI patients' plasma with rhLCAT restores HDL ability to promote endothelial NO production, possibly related to significant modification in HDL phospholipid classes.

Conclusions—Impairment of cholesterol esterification may be a major factor in the HDL dysfunction observed during acute coronary syndrome. rhLCAT is able to restore HDL-mediated NO production in vitro, suggesting LCAT as potential therapeutic target for restoring HDL functionality in acute coronary syndrome.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2019;39:915-924. DOI: 10.1161/ATVBAHA.118.311987.)**

Key Words: acute coronary syndrome ■ cholesterol acyltransferase ■ endothelial cell ■ lipoproteins ■ nitric oxide

Large epidemiological studies have clearly shown that low plasma HDL-C (high-density lipoprotein-cholesterol) levels are associated with an increased risk of cardiovascular events.[1–3] However, recent clinical and Mendelian randomization trials have doubts on the causal role of HDL (high-density lipoprotein) in atherosclerosis.[4,5] Among HDL functions, the best known is the ability to promote the removal of cholesterol from macrophages and shuttle it to the liver for excretion in the bile and feces.[6] Besides its role in cholesterol efflux and reverse cholesterol transport, HDL can contribute to the maintenance of vascular endothelium function through a variety of effects on vascular tone, inflammation, and endothelial cell (EC) homeostasis, and integrity.[7] In particular, HDL promotes nitric oxide (NO) release from cultured human ECs. Several mechanisms have been proposed to explain this effect, including cholesterol efflux, mediated by ABCA1 (ATP-binding cassette subfamily A member 1) and ABCG1 (ATP-binding cassette subfamily G member 1), which decreases the cholesterol content in caveolae, relieving the inhibitory interaction of eNOS (endothelial nitric oxide synthase) with caveolin-1, ABCG1-mediated 7-ketocholesterol efflux to HDL, suppressing the accumulation of reactive oxygen species that induce uncoupling of eNOS into inactive monomers, and SR-BI (scavenger receptor class B member 1)-mediated HDL-induced phosphorylation of eNOS at Ser1177, stimulating eNOS activation.[8] S1P (sphingosine-1-phosphate) has been

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Nonstandard Abbreviations and Acronyms

ABCA1	ATP-binding cassette subfamily A member 1
ABCG1	ATP binding cassette subfamily G member 1
ACS	acute coronary syndrome
apoA-I	apolipoprotein A-I
apoA-II	apolipoprotein A-II
apoB	apolipoprotein B
apoM	apolipoprotein M
CE	cholesteryl esters
CETP	cholesteryl ester transfer protein
ECs	endothelial cells
eNOS	endothelial nitric oxide synthase
HDL	high-density lipoprotein
HDL-C	HDL-cholesterol
LCAT	lecithin:cholesterol acyltransferase
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LpA-I	HDL containing only apoA-I
LpA-I:A-II	HDL containing both apoA-I and apoA-II
LPC	lysophosphatidylcholine
NO	nitric oxide
PC	phosphatidylcholine
PC/LPC	ratio of phosphatidylcholine to lysophosphatidylcholine
PON-1	paraoxonase 1
rHDL	reconstituted HDL
rhLCAT	recombinant human LCAT
S1P	sphingosine-1-phosphate
SAA	serum amyloid A
SR-BI	scavenger receptor class B member 1
STEMI	ST-segment–elevation myocardial infarction

shown to play a major role in HDL-induced NO-dependent vasorelaxation,[9,10] and apoM (apolipoprotein M), which is the physiological carrier protein for S1P in HDL, is needed to deliver S1P to ECs.[11]

Recent evidence suggests that the vasoprotective effects of HDL are impaired in patients during an acute coronary syndrome (ACS). In particular, HDL from ACS patients are defective in promoting NO release from cultured ECs and also in inhibiting cell adhesion molecules expression.[12,13] The mechanisms behind this HDL dysfunction are likely various. A reduced activity of the enzyme PON-1 (paraoxonase 1), and a consequent elevation of malondialdehyde content in HDL, has been proposed to play a crucial role in the defective endothelial effect of HDL.[12] Furthermore, it has been shown that HDL from ACS patients, but not HDL from healthy controls, suppresses EC NO release by stimulating the scavenger receptor Lox-1 and that addition of an anti–Lox-1 neutralizing antibody rescues the ability of patient HDL to promote endothelial NO release.[12]

HDLs are highly heterogeneous and are continuously converted in plasma through the action of several enzymes and transfer proteins,[14] and it is still unclear which HDL subspecies may be responsible for specific vasoprotective properties. LCAT (lecithin:cholesterol acyltransferase) is the enzyme responsible for cholesterol esterification in plasma and plays a key role in HDL maturation and remodeling.[15] Few studies have suggested that LCAT activity is decreased during an acute myocardial infarction,[16,17] but the possible link between LCAT concentration/activity and the HDL dysfunction observed in ACS has never been tested. The present study was thus designed to evaluate changes in LCAT concentration and activity during ST-segment–elevation myocardial infarction (STEMI) and to investigate if these changes are related to the compromised HDL capacity to promote endothelial NO production in ACS patients.

Materials and Methods

The raw data that support the findings of this study are available from the corresponding authors upon reasonable request.

Patient Selection

Patients were considered eligible if they were adults (≥18 years) and presented at clinical evaluation within a STEMI, defined according to the current European Society of Cardiology guidelines. The exclusion criteria were as follows: (1) late presentation (>12 hours after the onset of symptoms); (2) cardiogenic shock at admission (Killip class >3); and (3) lack of consent. Laboratory examinations, anthropometric parameters, clinical history, and ECG findings were collected. The study conformed to the guidelines set out in the Declaration of Helsinki and was approved by the internal ethics committee (Approval no 257-052014). All patients provided written informed consent.

Biochemical Analyses

Blood samples were collected in EDTA tubes at admission, 48 and 72 hours thereafter, at hospital discharge, and at 30-day follow-up. At all time points, plasma levels of total and HDL cholesterol, unesterified cholesterol, and triglycerides were determined by certified enzymatic techniques. LDL-C (low-density lipoprotein-cholesterol) was calculated by the Friedewald's formula. ApoA-I (apolipoprotein A-I), apoA-II (apolipoprotein A-II), and apoB (apolipoprotein B) levels were determined by immunoturbidimetry, using commercially available polyclonal antibodies. The plasma concentration of LpA-I (HDL particles containing only apoA-I) and of LpA-I:A-II (particles containing both apoA-I and apoA-II) was determined by electroimmunodiffusion in agarose gel (Sebia Italia). Serum content of pre-β-HDL was assessed by (2-dimensional)-electrophoresis followed by immunodetection with commercial polyclonal antibodies against human apoA-I.[18]

Plasma LCAT concentration was measured by immunoenzymatic assay,[19] and LCAT activity was determined using an exogenous standardized substrate as previously described.[20] Cholesterol esterification in plasma was assessed by calculating the unesterified/total cholesterol ratio.[20] Plasma PON concentration was measured by immunoenzymatic assay (Cusabio), and plasma PON arylesterase activity was measured by spectrophotometry at 270 nm in a reactions mixtures composed of 3.4 mmol/L phenylacetate (Sigma-Aldrich), 9 mmol/L Tris hydrocholoride, pH 8, and 0.9 mmol/L calcium chloride at 24°C.[21] Plasma SAA (serum amyloid A), apoM, and CETP (cholesteryl ester transfer protein) concentration were measured by immunoenzymatic assay (Invitrogen and Cloud Clone Corp).

Plasma Phospholipid Content

Plasma samples were subjected to 2 consequent extractions (chloroform/methanol 2:1; KCl 0.05%) and the organic phase loaded onto a multichannel high-performance thin-layer chromatography (HPTLC) plate (Whatman). Phospholipid classes were separated by thin-layer chromatography.[22] The amount of phosphorus in phospholipids was estimated after reading with a spectrophotometer (BioRad) at a 750-nm wavelength, by summing the absorbance of all the subclasses (minus the corresponding blank values), and calculating the relative percentages. The ratio of phosphatidylcholine to lysophosphatidylcholine (PC/LPC) was also obtained from the same absorbance data.

HDL Purification

HDLs (d=1.063–1.21 g/mL) were separated by sequential ultracentrifugation from plasma collected from all patients at admission, 48 and 72 hours after event, at hospital discharge, and at 30-day follow-up. After separation, HDLs were frozen at −80°C and dialyzed against sterilized saline immediately before use for studies on ECs.

Studies on ECs

HDLs were incubated for 30 min in primary cultures of human umbilical vein ECs (Clonetics, Lonza) at a protein concentration of 1.0 mg of protein per milliliter. After incubation, NO production was measured using a diacetate derivative of 4,5-diaminofluorescein (Sigma-Aldrich Chemie) as previously described.[13] Fluorescence intensity was detected using a Synergy Multi-Mode microplate reader equipped with the GEN5 software (BioTek), and data were normalized by total protein concentration in the cell lysate. To assess if NO production was mediated by eNOS, eNOS activation by phosphorylation was evaluated by SDS-PAGE and immunoblotting after 10 min of incubation with HDL. Membranes were developed against phosphorylated eNOS (Ser1177, Cell Signalling Technology), stripped, and reprobed with an antibody against total eNOS. Bands were visualized by enhanced chemiluminescence (GE Healthcare Biosciences) and analyzed with a GS-690 Imaging Densitometer and Multi-Analyst software (Bio-Rad Laboratories) as described.[23]

In Vitro Studies With rhLCAT

Plasma (3.0 mL) from additional 7 STEMI patients collected 48 and 72 hours after admission was incubated with rhLCAT (recombinant human LCAT, MEDI6012, Medimmune; 150 units in 0.6 mL), or an equal volume of saline.[24] Plasma samples were incubated at 37°C for 6 hours and then placed on ice.

After incubation, plasma levels of total, unesterified, and HDL cholesterol were determined as described above; plasma cholesteryl esters (CE) were calculated as (total cholesterol−unesterified cholesterol)×1.68. Plasma lipoprotein profile was analyzed by fast performance liquid chromatography using a Superose 6 HR10/30 column (GE Healthcare, UK).[23] Samples of 400 µL of plasma were applied to the column; 0.9-mL fractions were collected; and total and unesterified cholesterol and phospholipid were determined by enzymatic methods in each fraction.

Plasma HDL were immediately isolated by ultracentrifugation at the end of the incubation, and their ability to promote NO production was assessed as described above. To determine HDL composition, protein content was measured by the method of Lowry and lipids were measured by enzymatic techniques. Protein composition of isolated HDL was assayed by SDS-PAGE, and the intensity of apoA-I, apoA-II, and SAA bands was analyzed with a GS-690 Imaging Densitometer and Multi-Analyst software (Bio-Rad Laboratories) and expressed as percentage of the total proteins. ApoM was measured by immunoenzymatic assay (Cloud Clone Corp). To evaluate HDL phospholipid composition, isolated lipoproteins were subjected to 2 consequent extractions (chloroform/methanol 2:1; KCl 0.05%) and the organic phase loaded onto a multichannel HPTLC plate (Whatman). Phospholipid classes were separated and analyzed as described earlier. To verify the phospholipid classes in HDL, sample volumes of 100 µL were extracted according to Bligh and Dyer[25] as reported[26] using di-heptadecanoylglycerophospholipid as internal standard. Samples were taken up in 500 µL and stored at −80°C. Fifty microliter of extract was dried down and then diluted with 200 µL of running solvent (chloroform/methanol/aq ammonium formate, 29/64/3.4 v/v/v). Samples were directly injected at 100 µL/min into an Agilent 6460 Triple Quadrupole mass spectrometer equipped with a JetStream source and detected in the positive ion mode using precursor ion analysis monitoring *m/z* 184.1 with the following settings: sheath gas temp =220°C, sheath gas flow =7 L/min, CE=20 V, fragmentor =125, acceleration voltage =7 V, capillary =3000 V, nozzle =1000 V. Fatty acid distribution in the phospholipids was determined in the negative ion mode using precursor ion scanning for each of the potential fatty acids, tetradecanoate to docosanoate, including the more common unsaturated fatty acids using the following settings: sheath gas temp =220°C, sheath gas flow =7 L/min, CE=40 V, fragmentor =125, acceleration voltage =7 V, capillary =3000 V, nozzle =1000 V.

S1P concentrations were measured using the method described by Berdyshev et al[27] with 20 pg/µL of C17S1P as the final concentration of the internal standard. After extraction and derivatization, bisacetylated S1P was eluted from an Agilent Eclipse XDB-LB column (4.6 mm×150 mm 5-µm particle size) and detected in the negative ion mode using an Agilent 6460 Triple Quadrupole mass spectrometer with a JetStream source. Bisacetylated sphingolipids were eluted using the following gradients: 2-min hold of solvent A, ramp to 100% solvent B over 3 min, hold at 100% solvent B for 3 min, and then regenerate the column with solvent A for 4 min with the following settings: sheath gas temp =325°C, sheath gas flow =11 L/min, CE=13 V, fragmentor =210, acceleration voltage =7 V, capillary =3500 V, nozzle =1000 V. Solvent A=water/methanol/formic acid (20/80/0.5 v/v/v) mmol/L in ammonium formate. Solvent B=methanol/acetonitrile/formic acid (60/40/0.5 v/v/v) 5 mmol/L in ammonium formate. Multiple reaction monitoring transitions monitored were C17S1P *m/z* 448/388 and S1P *m/z* 462/402.4.[28,29]

In Vitro Studies With Reconstituted HDL

Apolipoprotein A-I was purified from human plasma as previously described.[30] Discoidal rHDL (reconstituted HDL) containing apoA-I and L-α-palmitoyloleoylphosphatidylcholine were prepared by the cholate dialysis technique.[31] rHDL containing both L-α-lysophosphatidylcholine (LPC) and L-α-palmitoyloleoylphosphatidylcholine were prepared by adding LPC to total phospholipid solution (w/w) to mimic PC/LPC ratio detected in patients' HDL before and after incubation of plasma with rhLCAT. To verify phospholipid composition, rHDL were subjected to 2 consequent extractions and the organic phase loaded onto a multichannel HPTLC plate as described above. Protein content was measured by the method of Lowry.

Statistical Analyses

Categorical data are presented as frequency and percentage; continuous data are presented as mean±SD, unless otherwise stated. Changes versus baseline are presented as means±SE. Because of the non-normal distribution of some of the variables, changes versus baseline were tested by the Wilcoxon signed-rank test. Associations between variables were assessed by robust regression (SAS procedure ROBUSTREG), a method which is less sensitive to outliers then ordinary linear regression. Because of the limited sample size, we tested a maximum of 3 independent predictors in multivariable models. Two-sided *P* below 0.05 were considered as significant. All analyses were performed using SAS v 9.4.

Results

Characteristics of Patients Population

From September 2014 and January 2016, a total of 30 patients were recruited into the study. The mean age was 66 years; 23.3% of the patients were woman; roughly one fourth of the patient population had a history of diabetes mellitus; 20% of the patients had experienced a prior myocardial infarction; and most patients had multiple cardiovascular risk factors (Table 1).

All patients underwent primary percutaneous coronary intervention. Periprocedural GPIIb/IIIa inhibitors were used in 60% of percutaneous coronary intervention, and at least 1 everolimus-eluiting or zotarolimus-eluiting stent or bare metal stent was deployed in 100% of the patients.

The characteristics of the patients at baseline are summarized in Table 1. Mean plasma total and LDL (low-density lipoprotein) cholesterol, apoB, and triglyceride levels were

Table 1. Characteristics of the Study Population

Characteristics	
Demographics	
n	30
Age, y	66±10
Sex, M/F	23/7
BMI, kg/m^2	27±4
Hypertension, %	73.3
Hypercholesterolemia, %	56.7
Current smoker, %	33.3
Obesity, %	23.3
Diabetes mellitus, %	23.3
Clopidogrel, %	6.7
Aspirin, %	26.7
Statin, %	43.3
Beta-blocker, %	66.7
ACE inhibitor or ARB, %	30
Laboratory parameters	
Total cholesterol, mg/dL	191.1±41.5
Unesterified cholesterol, mg/dL	50.9±11.4
LDL-cholesterol, mg/dL	121.9±33.3
HDL-cholesterol, mg/dL	40.0±11.3
Triglyceride, mg/dL	145.3±94.3
Apolipoprotein A-I, mg/dL	101.3±25.3
Apolipoprotein A-II, mg/dL	22.9±4.7
Apolipoprotein B, mg/dL	112.0±27.5

Data are expressed as mean±SD or as percentage where indicated. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; F, female; HDL, high-density lipoprotein; LDL, LDL, low-density lipoprotein; and M, male.

within the normal range, while HDL-C, apoA-I, and apoA-II levels tended to be low.

Total, LDL, and HDL cholesterol levels significantly and progressively decreased during STEMI, as already described in a similar patient population.[32] Plasma apoA-I, apoA-II, and apoB also significantly decreased during STEMI, while plasma triglyceride did not change. LpA-I concentration rapidly and significantly decreased, while LpA-I:A-II particles decreased slowly (Figure I in the online-only Data Supplement); pre-β-HDL did not change significantly during STEMI (Figure I in the online-only Data Supplement).

Changes in LCAT Concentration and Activity During STEMI

Plasma LCAT concentration significantly decreased within 48 hours (−0.49±0.11 μg/mL; P<0.001; Figure 1) and remained reduced at 72 hours. The reduction in LCAT concentration was paralleled by a significant reduction in LCAT activity (−3.15±1.05 nmol/mL per hour; P=0.006; Figure 1), with no changes in LCAT-specific activity. Plasma unesterified to total cholesterol ratio significantly increased during STEMI (Figure 1), clearly indicative of a compromised cholesterol esterification process. Interestingly, while LCAT mass and activity returned to values similar to admission at 30-day follow-up, the unesterified to total cholesterol ratio remained increased (Figure 1), thus suggesting that the cholesterol esterification process was still compromised.

Changes in PON, SAA, and ApoM During STEMI

Plasma PON concentration tended to decrease during STEMI but the reduction did not reach the statistical significance, while PON arylesterase activity was markedly reduced at 48 hours and returned to values similar to admission at 30-day follow-up (Figure 2). Plasma SAA concentration increased dramatically at 48 hours, remaining very high at 72 hours and returning to admission values at follow-up (Figure 2). Plasma apoM concentration significantly decreased at 48 hours, remaining significantly reduced at 72 hours and returning to admission values at follow-up (Figure 2). Plasma CETP concentration slightly but significantly increased at 72 hours (Figure 2).

Changes in Plasma Phospholipids During STEMI

Plasma PC/LPC ratio increased at 48 hours after admission, remained very high at 72 hours, and it returned to admission values at follow-up (Figure 3). Interestingly, and as expected by the LCAT reaction, the trend observed in PC/LPC ratio mirrors that observed for LCAT activity.

Effects of HDL Isolated During STEMI on Endothelial NO Production

The ability of HDL to promote NO production, tested in cultured ECs, progressively decreased during STEMI, reaching a minimum at 72 hours (−0.13±0.06 fold; P=0.04; Figure 4). Notably, HDL-induced NO production remained defective at 30-day follow-up (Figure 4). Similarly, HDL ability to induce eNOS activation progressively decreased during STEMI, reaching a minimum at 72 hours (−0.24±0.10 fold; P=0.027; Figure 4), thus suggesting that the observed reduction in endothelial NO production is due to impaired HDL-mediated eNOS activation.

Since changes in LCAT concentration/activity are anticipated compared with changes in HDL functionality, we also tested, by robust regression, the association between the various parameters at different time points. Notably, a significant positive correlation was observed between the reduction in HDL-mediated endothelial NO production at 72 hours and the reduction in LCAT concentration detected at 48 hours (R^2=0.20; P=0.002; Figure 5). No other independent predictor was detected.

Incubation of STEMI Patients' Plasma With rhLCAT

Since the reduction in plasma LCAT concentration was related to the extent of HDL dysfunction during STEMI, we tested if the addition of LCAT to patient plasma in vitro could restore HDL functionality. To test this hypothesis, blood samples from 7 additional STEMI patients (characteristics reported in

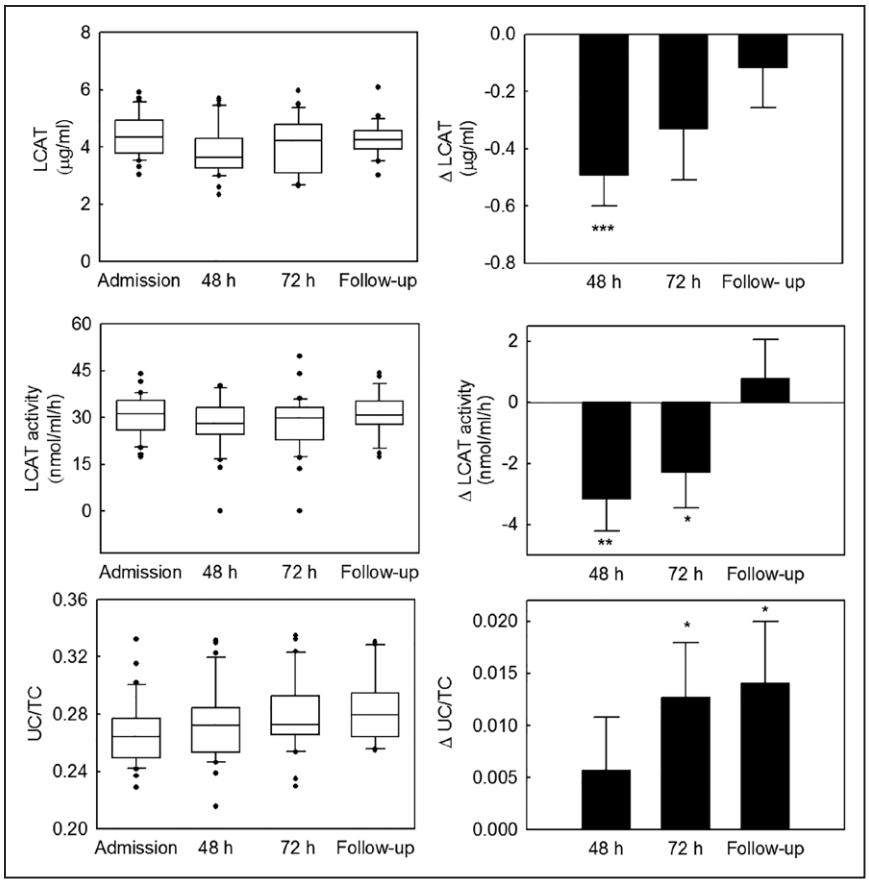

Figure 1. Cholesterol esterification during ST-segment–elevation myocardial infarction (STEMI)**.** LCAT (lecithin:cholesterol acyltransferase) concentration, LCAT activity, and unesterified/total cholesterol ratio (UC/TC) were measured in 30 STEMI patients at admission, 48 and 72 h thereafter, and at 30-day follow-up. Boxes indicate the median value with the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles, circles indicate values outside the 10th–90th percentiles. Bars indicate the mean±SEM of variation with respect to admission. **P*<0.05, ***P*<0.01, ****P*<0.001 vs admission.

Table I in the online-only Data Supplement) were collected 48 and 72 hours after admission and plasma was incubated at 37°C for 6 hours with rhLCAT or saline. As expected, the incubation with rhLCAT promoted the conversion of unesterified cholesterol into CE (Table II in the online-only Data Supplement); moreover, HDL-C levels significantly increased

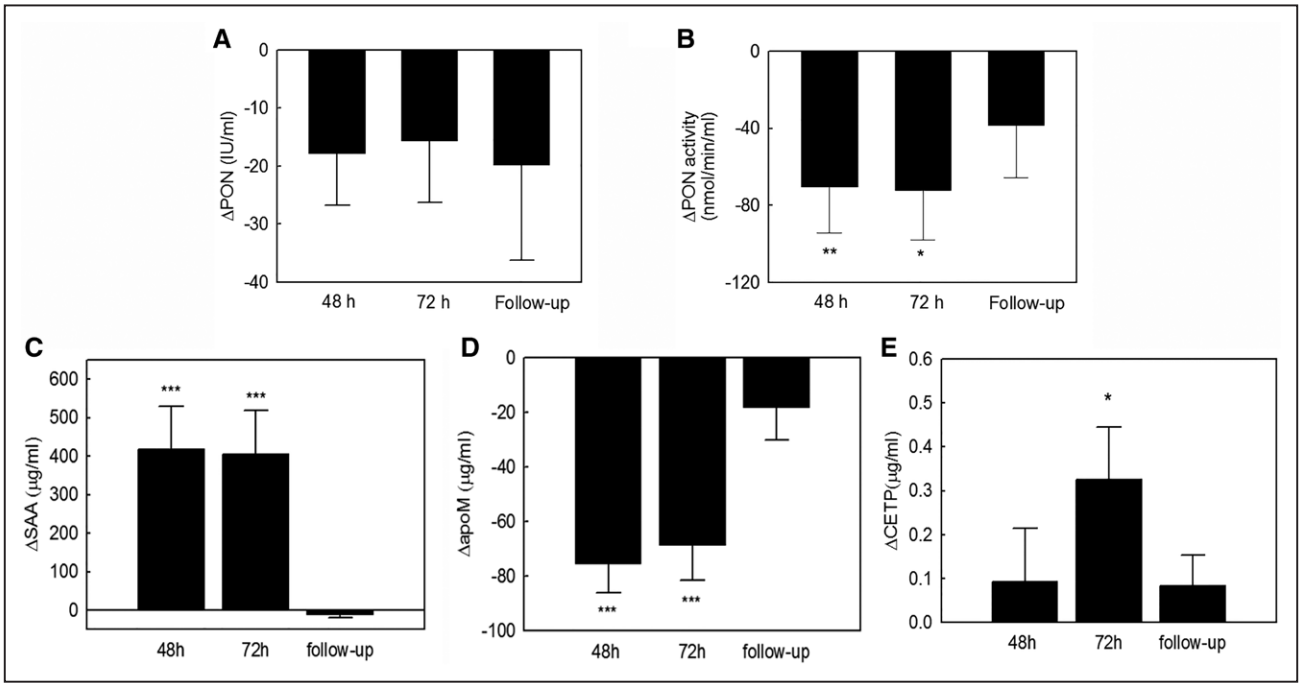

Figure 2. HDL (high-density lipoprotein)-associated proteins during ST-segment–elevation myocardial infarction (STEMI). PON (paraoxonase) concentration and activity and SAA (serum amyloid A), apoM (apolipoprotein), and CETP (cholesteryl ester transfer protein) concentration were measured in plasma of STEMI patients at admission, 48 and 72 h thereafter, and at 30-day follow-up. Bars indicate the mean±SEM of variation with respect to admission. **P*<0.05, ***P*<0.01, and ****P*<0.001 vs admission.

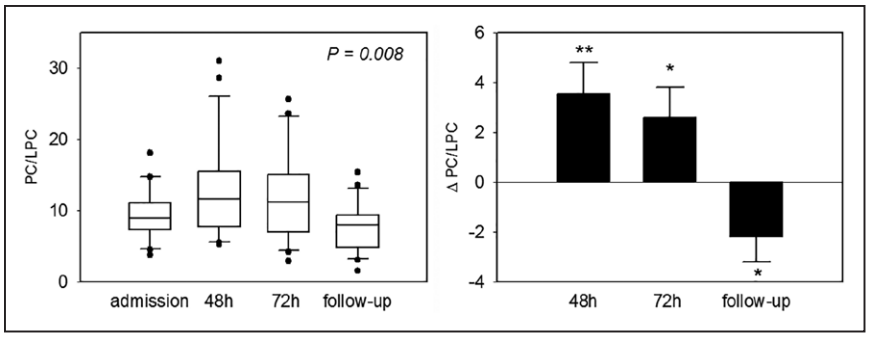

Figure 3. Plasma phospholipid changes during ST-segment–elevation myocardial infarction (STEMI). Phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) concentration was measured in 26 STEMI patients at admission, 48 and 72 h thereafter, and at 30-day follow-up. Results are expressed as PC/LPC ratio. Boxes indicate the median value with the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles, circles indicate values outside the 10th–90th percentiles. Bars indicate the mean±SEM of variation respect to admission. **P*<0.05, ***P*<0.01 vs admission.

and LDL-C decreased after incubation with rhLCAT (Table II in the online-only Data Supplement), likely explained by the increased CETP activity. Separation of lipoproteins by fast performance liquid chromatography showed that the incubation with rhLCAT led to reduction of phospholipid content both in HDL and LDL particles, while unesterified cholesterol was decreased in LDL and virtually disappeared in HDL (Figure II in the online-only Data Supplement).

When incubated with cultured ECs, HDL isolated from plasma incubated with rhLCAT proved to be more efficient than HDL isolated from the same plasma incubated with saline in promoting endothelial NO production (Figure 6). Notably, the ability to promote NO production of HDL isolated from plasma incubated with rhLCAT was similar to that we have shown for control HDL (1.29-fold as reported in Gomaraschi et al[33]).

The analysis of HDL composition after plasma incubation with rhLCAT showed a significant increase in CE and a significant decrease in unesterified cholesterol (Table 2), as shown in plasma. Moreover, changes were detected in specific protein and lipid components. HDL isolated after incubation with rhLCAT had a significantly decreased SAA content (−7.2%; *P*=0.026) and a significant increase in apoA-I (+3.8%; *P*=0.019) and apoM (+30%; *P*=0.001) content (Table 2). Interestingly, HDL isolated after incubation with rhLCAT, despite no other significant changes in total and single phospholipid masses, showed a decrease in PC and an increase in LPC content, which translates into a significant decreased PC/LPC ratio (from 15.16±1.77 to 6.95±0.89; Table 3), that becomes comparable to that observed in control HDL (6.41±1.16).

The analysis of ether PC showed that the predominant products were 1-O-alkyl-PC, with 1-O-alk-1-enyl PC constituting <0.9 mol% of the total PC. There was no significant difference in ether PC levels between HDL isolated after plasma incubation with rhLCAT versus saline (3.60±0.91 mol% and 3.58±1.19 mol%, respectively; *P*=0.934), likely ruling out a pivotal role of lipid oxidation. S1P content tended to increase, although not significantly, after incubation with rhLCAT (Table 3).

Finally, the fatty acid composition of PC analysis showed a general tendency toward a decrease in unsaturated fatty acids in HDL isolated after plasma incubation with rhLCAT (Table 3). This is an evidence of a restoration of LCAT activity because the enzyme specifically removes the fatty acid in sn-2 position, known to be mostly unsaturated. Indeed, the decrease in linoleic acid (c18:2w6) was also statistically significant (Table 3).

Synthetic HDL Containing LPC Promote Endothelial NO Production

To assess if the increased LPC content in HDL is implicated in their ability to promote endothelial NO production, rHDL with LPC content comparable to that observed in samples

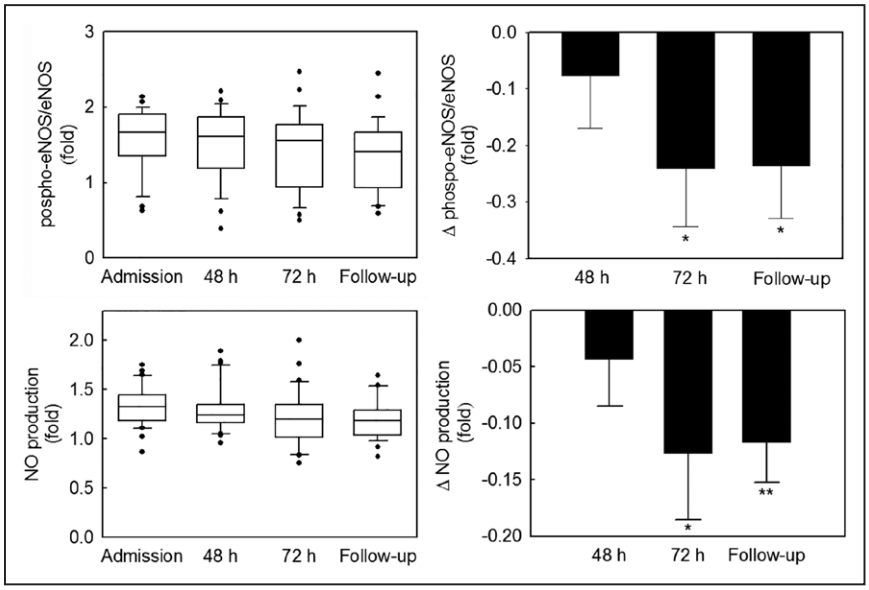

Figure 4. HDL (high-density lipoprotein)-mediated endothelial nitric oxide (NO) production during ST-segment–elevation myocardial infarction (STEMI). HDL were isolated from plasma from 30 STEMI patients collected at admission, 48 and 72 h thereafter, and at 30-day follow-up and incubated in human umbilical vein endothelial cell (HUVECs) to assess their ability to promote eNOS (endothelial nitric oxide synthase) activation by phosphorylation and NO production. Data are expressed as increased signal in treated vs untreated cells. Boxes display the median value with the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles, circles indicate values outside the 10th–90th percentiles. Bars indicate the mean±SEM of variation respect to admission. **P*<0.05, ***P*<0.01 vs admission.

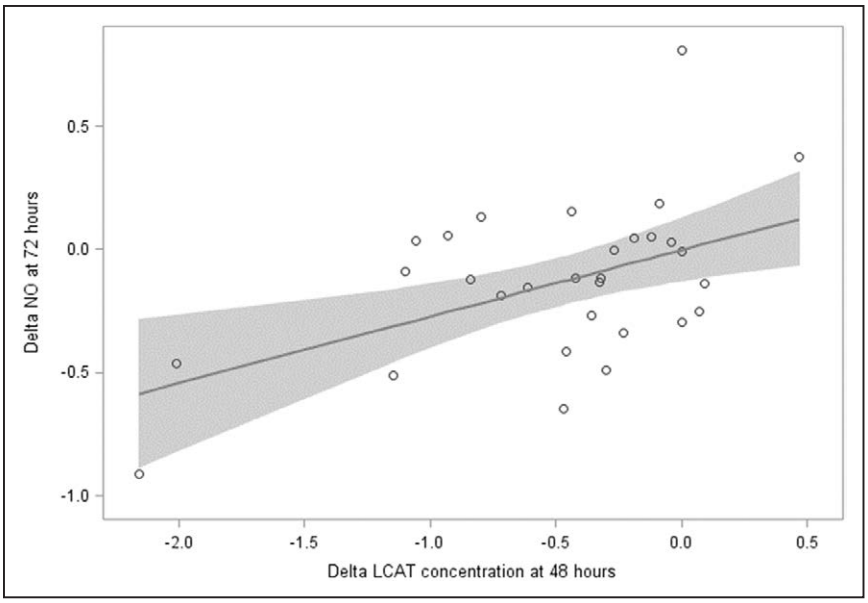

Figure 5. Correlation between HDL (high-density lipoprotein)-mediated endothelial nitric oxide (NO) production and LCAT (lecithin:cholesterol acyltransferase) mass. HDL capacity to promote endothelial NO production at 72 h after the event, expressed as delta vs admission, was plotted versus the LCAT mass at 48 h, expressed as delta vs admission. Association between variables was assessed by robust regression (R^2=0.20, P=0.002).

incubated with or without rhLCAT were prepared and incubated with ECs. rHDL with PC/LPC ratio of 7 proved to be more efficient in stimulating endothelial NO production compared with rHDL with lower amount of LPC (PC/LPC ratio of 15; Figure 7).

Discussion

The present study shows for the first time (1) that plasma LCAT concentration, and consequently activity, is reduced during ACS, (2) that HDL dysfunction observed in ACS is partly due to the reduction in LCAT concentration/activity, and (3) that in vitro incubation of ACS patients' plasma with rhLCAT fully restores HDL functionality.

HDL dysfunction in cardiovascular patients, including patients with ACS, diabetes mellitus, coronary artery disease, and chronic kidney disease, has been clearly demonstrated.[12,33–36] HDL isolated from cardiovascular patients are defective in promoting NO release from cultured ECs and also in inhibiting cell adhesion molecules expression.[12] The mechanisms behind this HDL dysfunction have been investigated, and various players have been proposed to be involved. Reduced activity of the enzyme PON-1, and a consequent elevation of malondialdehyde content in patient HDL, has been proposed to play a crucial role.[12] Here we show that the reduction in LCAT concentration observed early during ACS contributes to HDL dysfunction.

LCAT is the only enzyme able to esterify cholesterol in plasma through a transesterification reaction, in which occurs the conversion of cholesterol and PC to CE and LPC. LCAT also plays a major role in HDL remodeling and metabolism,[15] being responsible of the conversion of small discoidal HDL in large spherical HDL particles. Few studies have evaluated the LCAT system during ACS, mainly by measuring LCAT activity or plasma cholesterol esterification.[16,17] However, none of the previous studies measured plasma LCAT concentrations. In the present study, we have measured both LCAT concentration and activity and showed that the reduction in LCAT activity is due to a reduction in plasma LCAT concentration, with no change in enzyme-specific activity. These results confirm previous observations in animals and humans that LCAT is a negative acute-phase protein,[37–39] although possible interference with LCAT activity by substances induced during inflammation early in ACS such as myeloperoxidase cannot be ruled out.[17]

Chronic exposure to reduced LCAT concentration and activity, as observed in genetic LCAT deficiency, is associated with dramatic quantitative and qualitative alterations in HDL.[40] Carriers of LCAT deficiency have reduced HDL levels and abnormal HDL subclass distribution characterized by increased amounts of small discoidal HDL particles, absent or reduced large spherical particles,[40,41] and a selective depletion in LpA-I:A-II particles.[42] Contrary to that observed in genetic LCAT deficiency, the acute LCAT deficiency observed in ACS

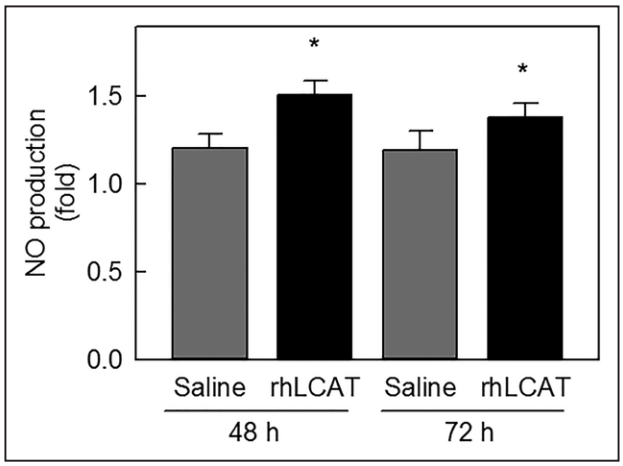

Figure 6. HDL (high-density lipoprotein)-mediated endothelial nitric oxide (NO) production after patients' plasma incubation with rhLCAT (recombinant human lecithin:cholesterol acyltransferase). Plasma from 7 ST-segment–elevation myocardial infarction (STEMI) patients collected 48 and 72 h after admission was incubated for 6 hours with rhLCAT or saline. HDLs were then purified and tested for their ability to promote endothelial NO production. Data are expressed as increased fluorescence in treated vs untreated cells, mean±SEM. *P<0.05 vs saline.

Table 2. HDL Composition After rhLCAT Incubation

	Saline	rhLCAT	*P* Value
UC, %	2.9±0.2	2.3±0.1	0.004
CE, %	15.2±0.8	18.9±0.7	0.001
PL, %	28.6±1.0	26.0±1.5	0.174
TG, %	4.5±0.4	5.0±0.7	0.564
Protein, %	48.3±0.9	47.8±1.3	0.782
ApoA-I, % of total protein	60.4±3.3	62.7±3.6	0.019
ApoA-II, % of total protein	3.3±0.4	3.2±0.4	0.596
SAA, % of total protein	32.9±3.5	30.7±4.0	0.026
ApoM, % of total protein	2.3±0.6	3.3±0.6	0.001

Data are expressed as mean±SEM. apoA-I indicates apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoM, apolipoprotein M; CE, cholesteryl ester; HDL, high-density lipoprotein; PL, phospholipid; rhLCAT, recombinant human lecithin:cholesterol acyltransferase; SAA, serum amyloid A; TG, triglyceride; UC, unesterified cholesterol.

is associated with a more significant reduction in apoA-I and LpA-I particles, leading to a HDL enrichment in LpA-I:A-II particles. Moreover, pre-β-HDL particles do not accumulate under acute reduction of LCAT, despite the previous observation of an inverse relationship between LCAT activity and pre-β-HDL in patients with ischemic heart disease.[43] These observations suggest that HDL particle phenotypic changes are different under acute and chronic LCAT suppression. The reduction in apoA-I and LpA-I under acute LCAT suppression suggests acute loss or catabolism of immature HDL particles, while the accumulation of pre-β-HDL under chronic LCAT suppression suggests adaptive changes that reduce loss of immature particles which, nevertheless, remain immature due to reduced LCAT activity and accumulate in the plasma. It is also worth mentioning that in ACS, other HDL-bound proteins involved in HDL remodeling and HDL functionality are modified. Among them are CETP, which decreases during ACS as shown in the present study and previously reported,[44,45] and apoM, which dramatically decreases early during ACS.

Table 3. HDL Phospholipid Composition

	Saline	rhLCAT	*P* Value
Phospholipids, mol%			
Phosphatidic acid, phosphatidylglycerol, and cardiolipin	1.15±0.24	1.52±0.33	0.377
Phosphatidylethanolamine	3.31±0.50	2.56±0.58	0.333
Phosphatidylinositol	1.70±0.51	3.09±0.85	0.172
Phosphatidylserine	2.08±0.63	2.41±0.62	0.707
PC	68.54±1.75	60.99±1.77	0.006
Sphingomyelin	18.02±0.70	18.92±1.13	0.507
LPC	5.19±0.57	10.49±1.42	0.002
PC/LPC	15.16±1.77	6.95±0.89	<0.001
Sphingolipids, pg/μg PC			
Sphinogosine-1-phosphate	0.68±0.04	0.82±0.07	0.088
Fatty acids on PC, mol%			
Arachidonic acid (20:4ω6)	7.53±4.31	5.96±3.53	0.259
Linoleic acid (18:2ω6)	26.29±6.80	22.26±5.66	<0.001
Oleic acid (18:1)	22.99±4.01	21.45±3.07	0.148

Data are expressed as mean±SEM (n=14). HDL indicates high-density lipoprotein; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; and rhLCAT, recombinant human lecithin:cholesterol acyltransferase.

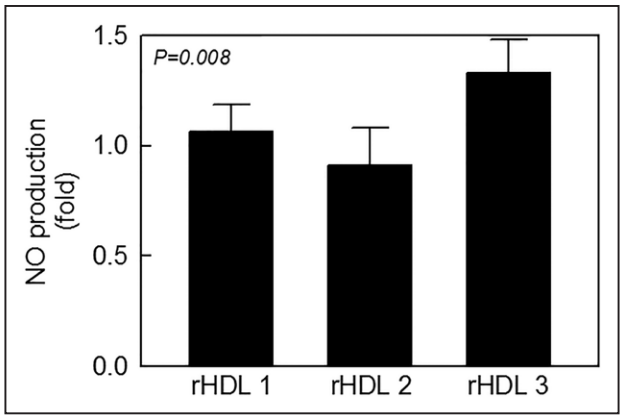

Figure 7. Effect of phospholipid composition on HDL (high-density lipoprotein)-mediated endothelial nitric oxide (NO) production. rHDL (reconstituted HDL) were prepared with different amount of phosphatidylcholine (PC) and lysophosphatidylcholine (LPC), specifically rHDL 1 containing only PC, rHDL 2 containing the amount of PC and LPC resembling the ratio observed in patient's HDL (PC/LPC=15), and rHDL 3 containing the amount of PC and LPC resembling the ratio observed in patient's HDL after plasma incubation with rhLCAT (PC/LPC=7). The effect of phospholipid composition on the ability of rHDL to promote endothelial NO production was tested in human umbilical vein endothelial cell (HUVEC); one-way ANOVA *P* is reported. Data are expressed as increased fluorescence in treated vs untreated cells, mean±SEM. rhLCAT indicates recombinant human lecithin:cholesterol acyltransferase.

The present study tested the hypothesis that HDL modifications induced by the acute partial LCAT defect are responsible for the HDL dysfunction observed during the ACS. To test this hypothesis, we first evaluated the relation between the early observed LCAT drop and the later observed HDL dysfunction. The results clearly show that the acute suppression of LCAT observed within the first 48 hours after STEMI is a determinant of the reduced capacity of HDL to promote endothelial NO production observed 24 hours later. The causality of the relation was confirmed by the demonstration that treatment of HDL from ACS patients with rhLCAT ex vivo fully restores the capacity of HDL to promote endothelial NO production. It thus appears that modifications induced by LCAT modulate the capacity of HDL to promote endothelial protection. An obvious modification in lipids determined by LCAT is the conversion of phosphatidylcholine to LPC, leading to a decrease in PC content and an increase in LPC, and a consequent increased LPC/PC ratio in HDL after treatment of plasma with rhLCAT. While LPC could be toxic at high concentrations,[46] small amounts of LPC have been shown to stimulate eNOS expression[47,48] and induce SR-BI expression.[49] Moreover, a recent study has shown that decreased HDL-PC content is associated with an increased HDL functionality,[50] as confirmed by our results with reconstituted HDL particles. Plasma incubation with rhLCAT also promoted positive changes in protein

composition of HDL, which lose SAA, displaced by apoA-I, with no changes in apoA-II. Indeed, apoA-I being able to bind SR-BI is a major player in HDL-mediated endothelial NO production,[23] while apoA-II and SAA have negative effects.[23,51] Moreover, HDL become enriched in apoM, a relevant protein in HDL-mediated endothelial protection. In our in vitro experiments, the apoM increase was not paralleled by a significant increase in S1P content after plasma incubation with rhLCAT; however, because apoM is the physiological carrier of S1P, it cannot be excluded that in vivo a parallel increase in S1P could occur.

Plasma HDL carry a number of antioxidant molecules besides LCAT, and among them, PON-1 seems very relevant in some atheroprotective functions. The present findings confirmed that PON-1 is decreased during the early phase of ACS, as previously reported.[12] PON-1 reduction also contributes to the HDL dysfunction, although at a lesser extent than LCAT in our patient population.

Taken together, the present findings highlight for the first time the important role of the LCAT enzyme in determining HDL functionality during ACS, thus posing the basis for the potential use of drugs aimed at increasing LCAT concentration/activity in the setting of ACS.

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Disclosures

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Highlights

- Plasma LCAT (lecithin:cholesterol acyltransferase) concentration and activity are reduced during acute coronary syndrome.
- LCAT reduction is a determinant of HDL (high-density lipoprotein) dysfunction observed in acute coronary syndrome.
- Treatment of HDL from acute coronary syndrome patients with rhLCAT (recombinant human LCAT) ex vivo fully restores the capacity of HDL to promote endothelial nitric oxide production.