



Plasma FA composition in familial LCAT deficiency indicates SOAT2-derived cholesteryl ester formation in humans

Chiara Pavanello¹, Alice Ossoli¹, Arianna Strazzella¹, Patrizia Risè², Fabrizio Veglia³, Marie Lhomme⁴, Paolo Parini⁵, and Laura Calabresi^{1*}

¹Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, and ²Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Milano, Italy; ³Maria Cecilia Hospital, Cotignola, Italy; ⁴ICAN analytics, IHU ICAN Foundation for Innovation in Cardiometabolism and Nutrition, Paris, France; ⁵Cardio Metabolic Unit, Department of Medicine and Department of Laboratory Medicine, Karolinska Institutet, and Medicine Unit Endocrinology, Theme Inflammation and Ageing, Karolinska University Hospital, Stockholm, Sweden

Abstract Mutations in the *LCAT* gene cause familial LCAT deficiency (Online Mendelian Inheritance in Man ID: #245900), a very rare metabolic disorder. LCAT is the only enzyme able to esterify cholesterol in plasma, whereas sterol O-acyltransferases 1 and 2 are the enzymes esterifying cellular cholesterol in cells. Despite the complete lack of LCAT activity, patients with familial LCAT deficiency exhibit circulating cholesteryl esters (CEs) in apoB-containing lipoproteins. To analyze the origin of these CEs, we investigated 24 carriers of LCAT deficiency in this observational study. We found that CE plasma levels were significantly reduced and highly variable among carriers of two mutant *LCAT* alleles (22.5 [4.0–37.8] mg/dl) and slightly reduced in heterozygotes (218 [153–234] mg/dl). FA distribution in CE (CEFA) was evaluated in whole plasma and VLDL in a subgroup of the enrolled subjects. We found enrichment of C16:0, C18:0, and C18:1 species and a depletion in C18:2 and C20:4 species in the plasma of carriers of two mutant *LCAT* alleles. No changes were observed in heterozygotes. Furthermore, plasma triglyceride-FA distribution was remarkably similar between carriers of LCAT deficiency and controls. CEFA distribution in VLDL essentially recapitulated that of plasma, being mainly enriched in C16:0 and C18:1, while depleted in C18:2 and C20:4. Finally, after fat loading, chylomicrons of carriers of two mutant *LCAT* alleles showed CEs containing mainly saturated FAs. ■ This study of CEFA composition in a large cohort of carriers of LCAT deficiency shows that in the absence of LCAT-derived CEs, CEs present in apoB-containing lipoproteins are derived from hepatic and intestinal sterol O-acyltransferase 2.

Supplementary Key words familial LCAT deficiency • cholesteryl esters • lipoproteins • SOAT2 • cholesteryl ester FAs • VLDL • CEFA • apoB-containing lipoproteins • fat loading

Cholesteryl esters (CEs) are synthesized in humans by three distinct enzymes, LCAT, sterol O-1 acyltransferase (SOAT1), and sterol O-2 acyltransferase (SOAT2)—previously denominated ACAT1 and ACAT2. LCAT acts in plasma and other biological fluids, where it catalyzes the transacylation of the sn-2 FA of lecithin, which usually is polyunsaturated, to the free 3-OH group of cholesterol, generating CE and lysolecithin (1). The majority of circulating LCAT is bound to HDLs, whereas a minority is bound to apoB-containing lipoproteins (1). ApoA-I is the best LCAT activator, but other apolipoproteins (i.e., apoA-II and apoA-IV) can also activate the enzyme (1).

SOAT1 and SOAT2 are intracellular enzymes, encoded by two distinct genes (*SOAT1* and *SOAT2*), and have distinct tissue distribution and functions (2). SOAT1 is expressed in almost all cell types, including the Kupffer cells in the liver, and acts to prevent free cholesterol-induced cytotoxicity (3). SOAT2 is exclusively expressed in hepatocytes and enterocytes (4). Here, it participates to the regulation of intracellular cholesterol homeostasis and to the synthesis of CEs fated to apoB-containing lipoprotein secretion (VLDL and chylomicrons). The most commonly SOAT2-derived CEs are cholesteryl oleate (C18:1) and cholesteryl palmitate (C16:0) (5). Immediately after entering the circulation, VLDL will become substrate of LCAT, which will produce the majority of circulating CEs. Despite the unchanged or increased levels of apoB in circulation, *Soat2* deficiency in mice prevents atherosclerosis because of decreased content in VLDL-CEs and LDL-CEs and to a shift of lipoprotein cholesteryl ester FA (CEFA) composition toward the unsaturated forms (3, 6). In addition, a successful protection from atherosclerosis was also obtained by decreasing hepatic SOAT2 activity with an antisense oligonucleotide targeting *Soat2* mRNA in mice (7).

*For correspondence: Laura Calabresi, laura.calabresi@unimi.it.

Mutations in the gene coding for the LCAT enzyme cause the very rare recessive metabolic disorder called familial LCAT deficiency (FLD, Online Mendelian Inheritance in Man ID: #245900). Patients with FLD have severe HDL deficiency, hypertriglyceridemia, and an increased unesterified to total cholesterol ratio (8). In a recent analysis of the large Italian cohort of patients with FLD (8), we have shown that despite the absence of LCAT activity, carriers have circulating CEs, highly variable among subjects and of unknown origin. In the present study, we have evaluated the origin of circulating CEs in carriers of LCAT deficiency, testing the hypothesis that SOAT2 can contribute to the level of plasma CEs in humans.

MATERIALS AND METHODS

Study population

Twenty-four carriers of *LCAT* gene mutations, including eight carriers of two mutant *LCAT* alleles and seven heterozygotes, and nine noncarrier family members, all belonging to the Italian LCAT-deficient families (9, 10), were included in this observational study. Selection of subjects was conditioned by plasma sample availability. A detailed overview of the *LCAT* mutations can be found in [supplemental Table S1](#). All procedures were followed in accordance with the ethical standards of the local institutional committees on human experimentation and according to the Helsinki Declaration of 1964, as revised in 2013. The study was approved by the institutional Ethic Committees (approval number: 446-092014). All subjects were fully informed about the modalities of the study and enrolled after signed informed consent.

Plasma biochemical analyses

Blood samples were collected after an overnight fast in Na₂-EDTA tubes, plasma was separated by low-speed centrifugation at 4°C within a few minutes from collection, and then immediately frozen. Plasma total and HDL cholesterol, unesterified cholesterol (UC), triglyceride (TG), phospholipid (PL), and apolipoprotein levels were determined with certified methods using a Roche c311 autoanalyzer (Roche Diagnostics) (11). The amount of CEs was calculated by subtracting UC from total cholesterol, and the difference was multiplied by 1.68 to have a precise estimation of the CE mass. LDL and non-HDL cholesterol were calculated by the Friedewald's equation and as the difference between total cholesterol and HDL-cholesterol, respectively. In the case of TG levels >400 mg/dl, LDL cholesterol was assessed using a direct measurement method. Plasma CE and PL FA composition (CEFA, PLFA) was measured by a gas-liquid chromatographic method (12) in a representative group of subjects. Profile of FAs in plasma TG (TGFA) was determined by LC-MS/MS as previously described (13). TG species were detected as neutral loss of one FA chain. FA distribution was determined from molecular mass, FA chain loss, and retention time. Hepatic and intestinal SOAT2 contribution to CE formation was evaluated by analyzing CEFA composition in VLDL and chylomicrons, respectively, as described below.

Lipoprotein purification and analysis

Plasma lipoproteins for the composition analysis were separated by sequential ultracentrifugation using a Beckman

TL 100 ultracentrifuge equipped with a TL 100.3 rotor (Beckman Instruments, Fullerton, CA). Lipoproteins were dialyzed against saline to remove potassium bromide, and lipoprotein fractions were shown to be free of other lipoproteins by electrophoresis in agarose gel. Total cholesterol, UC, PL, and TG contents of lipoprotein fractions were determined by enzyme techniques; the CE mass was calculated as aforementioned. Protein content was measured by the method of Lowry using bovine serum albumin as standard.

VLDLs for the FA analysis were separated by fast-protein liquid chromatography using a single Superose 6 HR10/30 column (Pharmacia). Plasma samples (500 µl) were applied to the column and eluted in Tris buffer, and fractions were collected and pooled according to the elution times for VLDL, LDL, and HDL. Lipoprotein fractions were shown to be free of other lipoproteins by electrophoresis in agarose gel. Then, CEFA composition of isolated VLDL was determined as described aforementioned.

Test meal

Two homozygous FLD patients and two matched controls received, after an overnight fast, a test meal consisting of 2 eggs, 40 g butter, 40 g olive oil, 2 slices of bacon, and 2 salted brioches (1,478 kcal, fat 73% of total energy). The test meal was consumed during a period of 15 min, and the subjects were deprived of any source of energy for the next 6 h. Blood samples were collected in Na₂-EDTA (1 mg/ml) tubes before the test meal (0 h) and after 1, 2, 3, 4, and 6 h, and plasma was separated by low-speed centrifugation at 4°C. Plasma lipid/apolipoproteins were measured as described above. Chylomicrons were immediately separated from fresh plasma by ultracentrifugation at 50,000 rpm for 20 min at 4°C, using a Beckman TL 100 ultracentrifuge equipped with a TL 100.3 rotor (14). Plasma lipoproteins were separated by fast-protein liquid chromatography (see above). CEFA composition was determined in chylomicrons at 1, 2, 3, 4, and 6 h after meal as described above. Since CEFA distribution in plasma was not different to that of controls, high-fat load test was not performed in carriers of one mutant *LCAT* allele. Test meal nutrient composition is reported in [supplemental Table S2](#).

Statistical analysis

Data were summarized as median (first and third quartiles) and with *n* (%) for categorical variables, if not otherwise stated. The data were analyzed according to what were reported in figure and table legends. The number of samples for each test is reported in the figure or table.

The association of biochemical parameters with LCAT genotype was assessed by Spearman's rho correlation versus the number of mutant *LCAT* alleles (0, 1, or 2). Comparisons between groups were performed by Kruskal-Wallis nonparametric test followed by Dunn's correction for multiple comparisons or Mann-Whitney test, where appropriate.

GraphPad Prism 9 (GraphPad Software, Inc) was used for graphic presentation. All statistical analyses were performed using SPSS software, version 27.0 (SPSS, Inc, Chicago, IL). Tests were two-sided, and *P* values <0.05 were considered as statistically significant.

RESULTS

Lipoprotein composition is significantly altered in carriers of LCAT mutations

The characteristics of the subjects participating in the study are summarized in [Table 1](#). Carriers and controls

TABLE 1. Demographic and lipid/lipoprotein data in carriers of *LCAT* mutations and controls

Parameter	Carriers of two mutant <i>LCAT</i> alleles	Carriers of one mutant <i>LCAT</i> allele	Controls	<i>P</i>
<i>N</i>	8	7	9	
Gender (male/female)	5/3	3/4	4/5	
Age (y)	35.0 (33.0–37.8)	59.0 (32.0–60.0)	42.0 (31.5–74.5)	0.333
BMI (kg/m ²)	22.1 (20.0–26.8)	29.0 (22.5–30.4)	22.6 (20.8–25.8)	0.993
Total cholesterol (mg/dl)	162.5 (86.5–214.8)	164.0 (133.0–215.0)	226.0 (166.5–244.5)	0.046
UC (mg/dl)	144.7 (85.3–192.6)	43.0 (34.0–75.0)	49.0 (39.5–64.1)	<0.0001
UC/total cholesterol	0.90 (0.89–1.00)	0.32 (0.21–0.35)	0.26 (0.21–0.27)	<0.0001
CEs (mg/dl)	22.5 (4.0–37.8)	218.0 (153.0–234.0)	282.0 (216.0–317.5)	<0.0001
CEs/total cholesterol	0.17 (0.06–0.19)	1.15 (1.09–1.32)	1.25 (1.24–1.34)	<0.0001
Non-HDL cholesterol (mg/dl)	151.1 (73.0–209.8)	134.0 (100.0–169.0)	157.0 (108.0–195.5)	0.899
LDL cholesterol (mg/dl)	60.5 (47.0–143.0)	104.0 (78.0–135.0)	125.0 (91.5–155.5)	0.052
HDL cholesterol (mg/dl)	10.5 (7.0–15.8)	35.0 (30.0–45.0)	57.0 (48.5–68.5)	<0.0001
TGs (mg/dl)	307.5 (102.0–542.5)	144.0 (115.0–149.0)	97.0 (69.0–202.0)	0.042
PLs (mg/dl)	284.5 (190.5–375.8)	208.0 (171.0–256.0)	232.0 (174.0–244.5)	0.253
Apolipoprotein A-I (mg/dl)	36.0 (30.0–41.0)	107.0 (84.0–111.0)	132.0 (114.5–150.5)	<0.0001
Apolipoprotein A-II (mg/dl)	5.0 (5.0–9.00)	29.0 (26.0–35.0)	31.0 (29.0–36.0)	<0.0001
Apolipoprotein B (mg/dl)	51.0 (27.0–58.0)	94.0 (72.0–113.0)	102.0 (80.0–124.5)	0.005
LCAT activity (nmol/ml/h)	0.0 (0.0–0.0)	13.8 (7.4–23.1)	43.4 (26.6–49.5)	<0.0001
LCAT mass (µg/ml)	1.5 (0.9–2.3)	3.5 (2.9–4.8)	4.4 (3.2–5.3)	<0.0001

Data are reported as median (first and third quartiles) or numbers.
P values reported are calculated by Spearman's rho correlation.

were comparable for age and BMI. As previously reported (8), carriers of *LCAT* deficiency had lower LDL cholesterol and HDL cholesterol levels and higher TG than control subjects, whereas non-HDL cholesterol was similar in carriers and controls. Apolipoprotein B was significantly lower in carriers of two mutant *LCAT* alleles, making the non-HDL cholesterol/apolipoprotein B ratio much higher in carriers of two mutant *LCAT* alleles compared with heterozygous carriers and controls (2.38 [1.84–3.66], 1.41 [1.26–1.49], and 1.48 [1.40–1.55], respectively, *P* = 0.081). The TG/non-HDL cholesterol ratio was also significantly higher in carriers of two mutant *LCAT* alleles compared with heterozygous carriers and controls (2.33 [1.33–3.30], 1.11 [0.89–1.26], and 0.78 [0.62–1.08], respectively, *P* < 0.0001).

Consistently with the presence of pathogenic *LCAT* mutations, carriers showed significantly reduced *LCAT* mass and activity with a gene dose-dependent effect. Notably, *LCAT* activity was undetectable in all carriers of two mutant *LCAT* alleles, whereas in heterozygous carriers, it was half of controls. As a consequence of complete *LCAT* deficiency, cholesterol esterification was compromised in carriers (Table 1), and this was particularly evident in carriers of two mutant *LCAT* alleles. Indeed, they showed markedly increased plasma UC levels and unesterified/total cholesterol ratio. Surprisingly, CEs were detectable in plasma, although very low (88% lower compared with controls). As described for the entire Italian cohort (8), CEs in carriers of *LCAT* mutations display high variability, ranging from 4 to 87 mg/dl, and were significantly correlated with total cholesterol levels (*r* = 0.756; *P* = 0.030), independently of the causative mutation (supplemental Fig. S1). Consistently, CEs to total cholesterol ratio was significantly lower in carriers of two mutant *LCAT* alleles compared with controls (Table 1), without appreciable differences between females and males (0.13 [0.06] vs. 0.17

[0.06–0.23], respectively, *P* = 1.000). Carriers of one mutant *LCAT* allele had normal unesterified/total cholesterol ratio and plasma UC levels, whereas plasma CEs were slightly reduced compared with controls (Table 1).

The percentage of lipoprotein composition was measured in a representative subgroup of carriers and controls and reported in Figure 1. Lipoprotein composition was significantly affected by the presence of *LCAT* gene mutations (Fig. 1): both VLDL and LDL from carriers of two mutant alleles were characterized by an increased content of UC compared with control lipoproteins (9.3 ± 3.7% vs. 4.2 ± 1.4%, *P* < 0.001 for VLDL; 18.8 ± 3.9% vs. 8.9 ± 2.0%, *P* < 0.001 for LDL). Parallely, VLDL and LDL showed a significantly diminished content of CEs compared with controls (2.3 ± 2.1% vs. 8.4 ± 3.6%, *P* = 0.010 for VLDL; 5.2 ± 4.4 vs. 37.3 ± 5.1%, *P* < 0.0001 for LDL). CEs were almost undetectable in HDL from carriers of two mutant *LCAT* alleles (0.3 ± 0.7% vs. 21.5 ± 3.6%, *P* < 0.0001 vs. controls), suggesting that the little amount of circulating CEs in complete *LCAT* deficiency is carried only by apoB-containing lipoproteins. LDL from carriers of two mutant *LCAT* alleles displayed a very high content of PL compared with controls (*P* < 0.001, Fig. 1), confirming the presence of abnormal lipoproteins, that is, lipoprotein X (8), large vesicles that form to accommodate the excess of circulating UC and has a density similar to LDL. TG content was also increased in LDL from carriers of two mutant *LCAT* alleles (22.7 ± 6.1% vs. 8.6 ± 2.6% in control LDL, *P* < 0.0001).

The composition of VLDL from carriers of one mutant *LCAT* allele was almost identical to that of controls. Both carriers' LDL and HDL showed instead a higher content in TG than controls (14.9 ± 3.2% vs. 8.6 ± 2.6, *P* = 0.037 for LDL and 5.6 ± 1.1 vs. 3.4 ± 1.1, *P* = 0.011 for HDL).

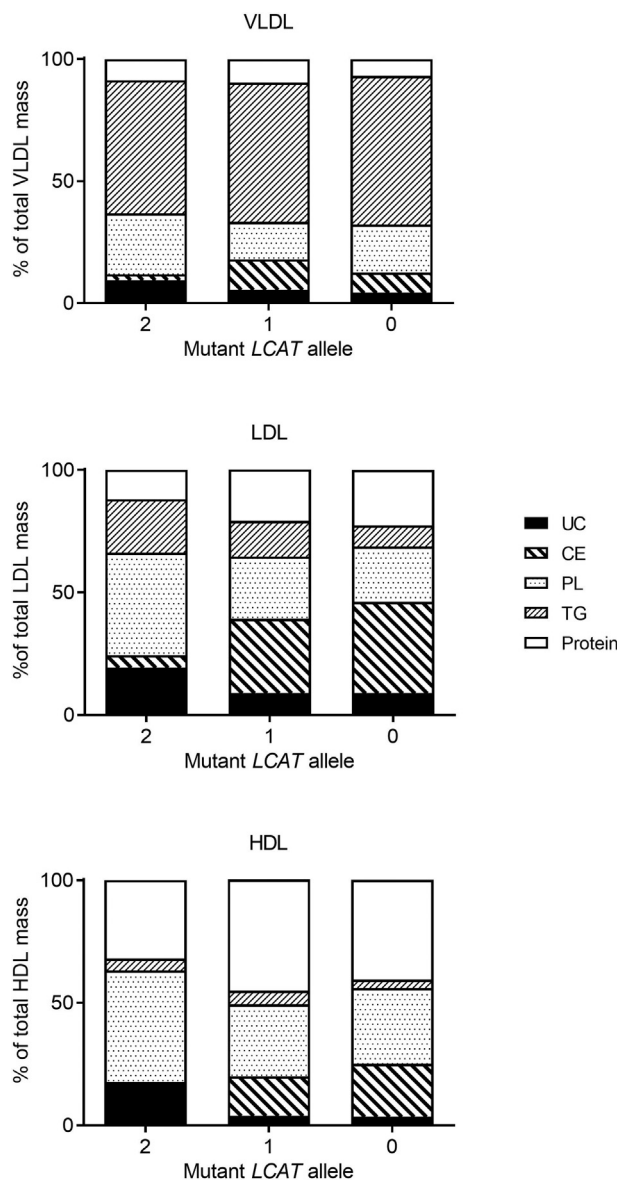


Fig. 1. Lipoprotein composition. Mass percentage composition of lipoproteins isolated by ultracentrifugation from the plasma of *LCAT* mutation carriers and controls. Data are expressed as mean, $n = 8$ for carriers of two mutant *LCAT* alleles, $n = 7$ for carriers of one mutant *LCAT* allele, and $n = 9$ controls.

CEFA composition in whole plasma and VLDL particles indicates the SOAT2 origin of circulating CEs

The percentage of FA composition of plasma CEs was measured, and the data are shown in [supplemental Table S3](#) and [Fig. 2](#). Plasma CEFA distribution showed an enrichment in saturated (C16:0 and C18:0) and monounsaturated (C18:1) FAs and a depletion in polyunsaturated FAs (C18:2, C20:4) in carriers of two mutant *LCAT* alleles. In addition, a significant increased plasma oleate/linoleate ratio (C18:1/C18:2) was observed compared with controls (4.71 [1.31–6.02] vs. 0.55 [0.51–0.60], $P < 0.002$). No significant changes were instead detected in carriers of one mutant *LCAT* allele.

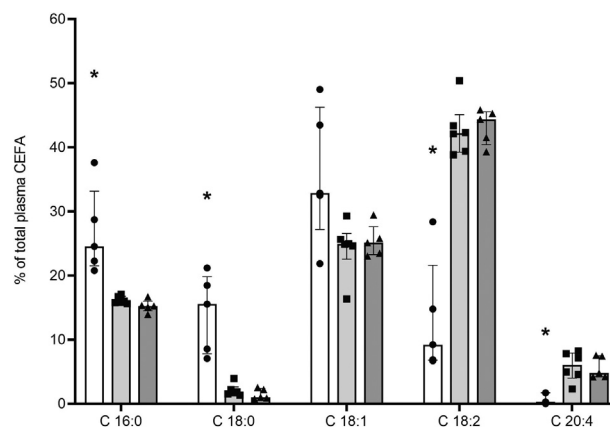


Fig. 2. Plasma CEFA composition. Scatter plot with aligned dot plot represents percentage composition of FA in CEs from plasma of *LCAT* mutation carriers and controls. Data are expressed as median (first and third quartiles), $N = 8$ for carriers of two mutant *LCAT* alleles (black circles, ●), $n = 7$ for carriers of one mutant *LCAT* allele (black squares, ■), and $n = 9$ controls (black triangles, ▲). * $P < 0.05$ versus controls. C16:0, palmitate; C18:0, stearate; C18:1, oleate; C18:2, linoleate; C20:4, arachidonate.

CEFA composition clearly indicates that they are SOAT2 derived. Both SOAT1 and SOAT2 use unsaturated and monounsaturated as preferential FA for CE formation, but only SOAT2 participates in the formation of CEs that are incorporated in apoB-containing lipoproteins (15). Thus, circulating CEs in *LCAT* deficiency are SOAT-2 derived.

To exclude a possible role of the diet, TGFA composition was also assessed and reported in [supplemental Table S4](#). Plasma TGFA distribution was remarkably similar between carriers of *LCAT* deficiency and controls ([supplemental Table S4](#)). PL FAs were also analyzed and showed only a reduced C20:4 content in carriers of two mutant *LCAT* alleles compared with controls ([supplemental Table S5](#)).

CEFA profile in VLDL essentially recapitulated that of plasma ([Table 2](#)). VLDLs from carriers of two mutant *LCAT* alleles were mainly enriched in C16:0 and C18:1, whereas were depleted in C18:2 and C20:4. The reduced content of C18:2 altered the CE oleate/linoleate ratio in VLDL: an increase of C18:1/C18:2 ratio was observed in subjects carrying two mutant *LCAT* alleles, consistent with what observed on whole plasma ([Table 2](#)). As observed in plasma, CEFA distribution in VLDL from carriers of one mutant *LCAT* allele was comparable to controls ([Table 2](#)).

CEFA composition in chylomicrons supports the SOAT2 origin of postprandial CE

To dissect the contribution of intestinal SOAT2 to CEs in circulation in the postprandial state, we analyzed FA composition of chylomicron CE (chylomicron CEFA) after a fat test meal. [Figure 3](#) reported chylomicron-CEFA composition at TG peak, which corresponded to 2 h after meal for control subjects and

TABLE 2. VLDL-CEFA distribution in carriers of *LCAT* gene mutations and controls

FA	Carriers of two mutant <i>LCAT</i> alleles	Carriers of one mutant <i>LCAT</i> allele	Controls	<i>P</i>
<i>N</i>	4	6	6	
VLDL				
C 16:0 (%)	26.7 (17.9–34.4)	15.2 (15.0–16.1)	18.9 (15.3–21.4)	0.018
C 18:0 (%)	0.0 (0.0–0.0)	0.6 (0.0–2.1)	0.0 (0.0–0.0)	0.079
C 18:1 (%)	49.5 (43.6–54.5) ^a	24.0 (17.6–25.9)	22.0 (20.5–23.6)	0.005
C 18:2 (%)	25.7 (20.0–27.5) ^a	51.3 (45.0–53.7)	49.2 (46.1–51.2)	0.005
C 20:4 (%)	0.0 (0.0–0.0) ^a	8.5 (4.9–9.4)	9.5 (8.8–10.7)	0.002
Total saturated (%)	26.7 (17.9–34.4)	15.8 (15.0–18.3)	18.9 (15.3–21.4)	0.068
Total monounsaturated (%)	49.5 (43.6–54.5) ^a	24.0 (17.6–25.9)	22.0 (20.5–23.6)	0.005
Total polyunsaturated (%)	25.7 (20.0–27.5) ^a	59.6 (57.6–65.1)	59.1 (56.4–62.8)	0.005

Data are reported as median (first and third quartiles). *P* values are for Kruskal-Wallis nonparametric test, followed by Dunn's correction for multiple comparisons.

^a*P* < 0.05 versus controls.

3 h after meal for carriers of two mutant *LCAT* alleles. In patients with *LCAT* deficiency, the percentage distribution of chylomicron CEs containing saturated, monounsaturated, or polyunsaturated FA was shifted toward the saturated ones, when compared with control chylomicrons (Fig. 3). The enrichment in palmitate (C16:0), stearate (C18:0), and oleate (C18:1) and the depletion in linoleate (C18:2) and arachidonate (C20:4) was consistent with what observed on whole plasma and in VLDL (supplemental Table S3 and Table 2). Chylomicron-CEFA distribution was almost unchanged with time after eating, both in carriers and controls (supplemental Fig. S2). Of note, CEFA distribution in FLD and controls were very similar in fasting and postprandial state, suggesting a comparable biochemical fingerprint of *SOAT2* in liver and intestine (Figs. 2 and 3).

DISCUSSION

The present study demonstrates that *i*) patients with familial *LCAT* deficiency have circulating plasma CEs, despite the complete absence of *LCAT* activity, which are only detected in apoB-containing lipoproteins but

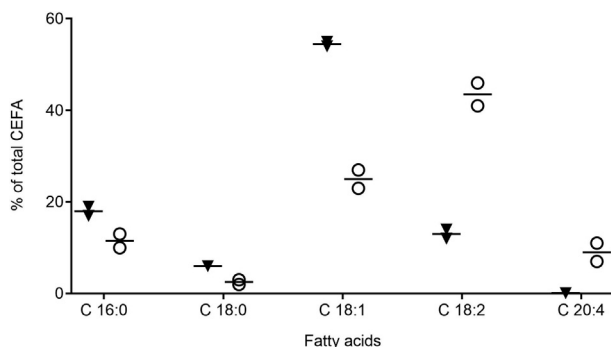


Fig. 3. CEFA composition of chylomicrons after high-fat meal. Aligned dot plots represent percentage composition of CEFA from plasma chylomicron of *n* = 2 *LCAT* mutation carriers (black triangles, ▼) and *n* = 2 controls (white circles, ○). Figure shows chylomicron CEFA at TG peak after high-fat meal. C16:0, palmitate; C18:0, stearate; C18:1, oleate; C18:2, linoleate; C20:4, arachidonate.

not in HDL and *ii*) the FA composition of circulating CEs indicates that they are *SOAT2* derived.

Familial *LCAT* deficiency is a very rare recessive disorder of lipoprotein metabolism caused by loss-of-function mutations in the *LCAT* gene (8). *LCAT* is the only enzyme able to esterify cholesterol in plasma, and around 70% of the circulating cholesterol is in the esterified form. The absence of *LCAT* thus has a dramatic impact on circulating lipoproteins, as clearly demonstrated by the lipoprotein composition reported in the present study. In patients with FLD, circulating HDLs have virtually no CEs and in fact are discoidal particles (16, 17), whereas VLDL and LDL contain a certain amount of CEs. The absence of CEs in carriers' HDL indicates that CE transfer protein does not operate lipid transfer between apoB-containing lipoproteins and HDL, likely because of the absence of core lipids in HDL.

The FA composition of VLDL-CEs demonstrates that they are produced by hepatic *SOAT2*. Cholesteryl oleate is largely predominant followed by cholesteryl palmitate (C16:0) in VLDL from patients with FLD, whereas in control VLDL, the most abundant is cholesteryl linoleate (C18:2), the enzymatic product of *LCAT*. These findings together with what observed in mice, where the deletion of both *Lcat* and *Soat2* genes leads to the complete absence of plasma CEs (15), support the concept that *SOAT2* is the enzyme producing CEs fated to secretion in lipoproteins.

Plasma levels of CEs are highly variable in carriers of two mutant *LCAT* alleles; this variability is not attributable to the type of *LCAT* mutation, since *LCAT* activity is completely absent in all patients with FLD, but is mainly proportional to total plasma cholesterol levels, which depends on several other genetic determinants. In our cohort, we did not observe a difference in circulating CEs between males and females, despite the lower microsomal activity of *SOAT2* observed in the liver of females in a small Chinese population (18). FA composition of chylomicron CEs isolated postprandially from carriers also shows to be *SOAT2* derived. Interestingly, FA distribution in chylomicrons does not change postprandially in carriers, confirming the complete absence of *LCAT* activity.

Several studies have provided evidence that the types of CEs that predominate in plasma contribute to the relative degree of atherogenicity. In the Atherosclerosis Risk in Communities study (19), carotid intima-media thickness was positively associated with the amount of circulating SOAT2-derived CEs (cholesteryl palmitate and cholesteryl oleate), independently of other cardiovascular risk factors. A large population-based prospective study (20) provided even more robust evidence in support of the atherogenicity of SOAT2-derived CEs, showing a significant positive association between the cholesteryl oleate and cholesteryl palmitate content of plasma lipoproteins and cardiovascular disease death. Conversely, cholesteryl linoleate, the typical product of LCAT, was inversely associated with cardiovascular disease death. In line with the evidence that polyunsaturated FA containing CEs derived from LCAT are associated with decreased atherosclerosis, whereas saturated and monounsaturated CEs derived predominantly from SOAT2 are associated with increased atherosclerosis, LCAT deficiency in mice resulted in more atherosclerosis (12). This does not apply in humans since carriers of familial LCAT deficiency have decreased preclinical atherosclerosis (21). It must be underlined that while mice lacking LCAT have still 50% of the circulating cholesterol in the esterified form (15), LCAT-deficient patients have only a little amount of CEs (21). Furthermore, mice genetically lacking LCAT (22) have unaltered plasma levels of apoB, whereas in LCAT-deficient patients, the levels of apoB are reduced. Hence, not only the quality of CEs but also the quantity and number of particles carrying them is relevant in atherogenesis.


Arterial plaque stabilization through the use of SOAT inhibitors has been investigated for two decades but unsuccessfully (23, 24). Failure of clinical trials have been attributed to the use of nonspecific SOAT2 inhibitors and to the consequently harmful effect of cell UC mediated by SOAT1 inhibition in macrophages (25). Whereas SOAT2 remains a potential therapeutic target for plasma cholesterol reduction and atherosclerosis prevention, targeting SOAT1 was found promising in other diseases, that is, Alzheimer's disease, where it is implicated in the amyloid plaque development (26).

Some limitations of our study should be appreciated. The number of analyzed FLD patients is small and has been limited by the availability of plasma samples. Subjects have been identified among the Italian cohort of LCAT-deficient carriers, which is a large cohort for a very rare dyslipidemia. In addition, females are very few in the entire cohort and only two in the present study. Finally, the postprandial evaluation was carried out in the only two FLD patients who agreed to participate in the study.

In conclusion, this is the first unequivocal demonstration that SOAT2 contributes CEs to newly secreted VLDL and chylomicrons in humans. The LCAT-

deficient model does not allow to address the potential role of SOAT2 inhibition in the treatment of atherosclerotic cardiovascular diseases, which remains to be proven.

Data availability

The data that support the findings of this study are available from the corresponding author on realistic request. 

Supplemental data

This article contains [supplemental data](#) (Tables S1–S5 and Figs. S1–S2).




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Author contributions

P. P. and L. C. conceptualization; C. P., A. O., A. S., P. R., F. V., and M. L. formal analysis; C. P. data curation; C. P., P. P., and L. C. writing—original draft; C. P., A. O., A. S., P. R., F. V., M. L., P. P., and L. C. writing—review & editing.

Author ORCIDs

Chiara Pavanello  <https://orcid.org/0000-0001-5892-9857>
Patrizia Risè  <https://orcid.org/0000-0002-7564-3508>
Fabrizio Veglia  <https://orcid.org/0000-0002-9378-8874>

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations

CE, cholesteryl ester; CEFA, cholesteryl ester FA; FLD, familial LCAT deficiency; PL, phospholipid; SOAT1, sterol O-1 acyltransferase; SOAT2, sterol O-2 acyltransferase; TG, triglyceride; TGFA, triglyceride FA; UC, unesterified cholesterol.

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