Role of LCAT in HDL remodeling: investigation of LCAT deficiency states

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Abstract To better understand the role of LCAT in HDL metabolism, we compared HDL subpopulations in subjects with homozygous (n = 11) and heterozygous (n = 11) LCAT deficiency with controls (n = 22). Distribution and concentrations of apolipoprotein A-I (apoA-I)-, apoA-II-, apoA-IV-, apoC-I-, apoC-III-, and apoE-containing HDL subpopulations were assessed. Compared with controls, homozygotes and heterozygotes had lower LCAT masses (−77% and −13%), and LCAT activities (−99% and −39%), respectively. In homozygotes, the majority of apoA-I was found in small, disc-shaped, poorly lipidated preβ-1 and α-4 HDL particles, and some apoA-I was found in larger, lipid-poor, discoidal HDL particles with α-mobility. No apoC-I-containing HDL was noted, and all apoA-II and apoC-III was detected in lipid-poor, preβ-mobility particles. ApoE-containing particles were more disperse than normal. ApoA-IV-containing particles were normal. Heterozygotes had profiles similar to controls, except that apoC-III was found only in small HDL with preβ-mobility. Our data are consistent with the concepts that LCAT activity: *1*) is essential for developing large, spherical, apoA-I-containing HDL and for the formation of normal-sized apoC-I and apoC-III HDL; and *2*) has little affect on the conversion of preβ-1 into α-4 HDL, only slight effects on apoE HDL, and no effect on apoA-IV HDL particles.—Asztalos, B. F., E. J. Schaefer, K. V. Horvath, S. Yamashita, M. Miller, G. Franceschini, and L. Calabresi. **Role of LCAT in HDL remodeling: investigation of LCAT deficiency states. *J. Lipid Res.* 2007.** 48: **592–599.**

Supplementary key words HDL subpopulations • apolipoproteins • reverse cholesterol transport

LCAT is a 416 amino acid protein that binds to lipoproteins or is present in lipid-free form in plasma and is secreted by the liver in humans (1). LCAT synthesizes the majority of cholesteryl esters in plasma by transferring a fatty acid from lecithin (phosphatidyl choline) to the 3-hydroxyl group of cholesterol. It is generally believed that LCAT maintains the unesterified cholesterol gradient between peripheral cells and HDL. Efflux of free cholesterol (FC) from cells occurs by a passive diffusion of FC between cellular membranes and acceptors and by mechanisms facilitated by scavenger receptor type B-I (SR-BI) and ABCs. In the presence of LCAT, the bi-directional movement of cholesterol between cells and HDL results in net cholesterol efflux (2, 3). Therefore, LCAT plays a central role in the initial steps of reverse cholesterol transport. LCAT is activated primarily by apolipoprotein A-I (apoA-I), but can also be activated by apoA-IV, apoC-I, and apoE (4, 5). Both the binding and activation of LCAT on the surface of HDL are essential for esterification of FC and accumulation of cholesteryl esters in the core of HDL.

Familial LCAT deficiency (FLD) is characterized by the absence of LCAT activity and reduced HDL cholesterol (HDL-C) level in plasma. In affected individuals, LCAT is either absent or present but inactive in plasma (6). LCAT has two distinct substrates: HDL and LDL. LCAT activity on HDL is called α-activity, and LCAT activity on LDL is called β-activity (7, 8). Lack of α-LCAT activity causes fish eye disease (FED). Homozygous subjects with FLD have corneal opacification, anemia, proteinuria, hematuria, and ultimately, renal failure, often requiring kidney transplantation (9). FED subjects have no clinical manifestation other than an age-dependent corneal opacification. Although it is not clear whether LCAT deficiency is directly linked to premature coronary artery disease (CAD), increased risk for CAD has been reported in some patients (9). Data obtained from cholesterol-fed human-LCAT transgenic rabbits indicated that HDL-C increased

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Abbreviations: apoA-I, apolipoprotein A-I; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; FC, free cholesterol; FED, fish eye disease; FLD, familial LCAT deficiency; HDL-C, HDL cholesterol; sPLA2, secretory phospholipase A2; SR-BI, scavenger receptor type B-I; TG, triglyceride.

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due to decreased catabolism of larger HDL particles, suggesting that the size of HDL may modulate the selective HDL-C uptake by the liver (10). In human-LCAT transgenic mice, the liver uptake of HDL was reduced by 41%, resulting in a substantial increase of large HDL particles that might be atherogenic (11) due to the fact that mice lack cholesteryl ester transfer protein (CETP) and that continued increase of cholesteryl ester in HDL by high levels of LCAT changes both the size and lipid composition of HDL. When CETP was coexpressed in LCAT transgenic mice, HDL size and composition changed and the animals were protected from atherosclerosis (12). These data suggest that under normal conditions in which CETP is present as in humans, increased LCAT activity is likely to increase HDL cholesterol and size and might reduce the risk for atherosclerosis. Our previous data suggest that the two largest, spherical, cholesteryl ester-rich HDL particles, α-1 and α-2, are good substrates for SR-BI in a human hepatoma cell line (13).

Our aim was to gain insight into the role that LCAT plays in HDL metabolism as well as to better understand LCAT deficiency states. We have examined apoA-I-, -A-II-, -A-IV-, -C-I-, -C-III-, and -E-containing HDL subpopulation profiles in LCAT-deficient homozygotes and heterozygotes and in control subjects. The data we present indicate that LCAT plays a very significant role in HDL particle metabolism, composition, and remodeling.

MATERIALS AND METHODS

Subjects

We examined plasma obtained from 11 homozygous LCAT-deficient subjects of Italian (n = 7), Japanese (n = 3), and US (n = 1) origin, as well as from 11 heterozygous LCAT-deficient subjects from Italy. Plasma obtained from gender-matched control subjects from the US (n = 15), Italy (n = 4), and Japan (n = 3) was used in this comparison. Homozygous and heterozygous subjects from Italy have been described previously (14). All homozygous subjects had primary hypoalphalipoproteinemia as defined by a plasma HDL-C level below the 5th percentile for the age- and gender-matched general populations of the specific countries. One homozygous subject from Japan had FED; however, none of the measured parameters of this subject were different by more than 1 SD from those of the other 10 homozygotes.

Sample handling and measurements

Blood was collected from all subjects after an overnight fast and immediately placed on ice. Plasma was separated by low-speed centrifugation at 4°C and was stored at −°80 C until use. Samples were sent to the Lipid Metabolism Laboratory at Tufts University on dry ice and were thawed in a 37°C water bath for 1–2 min and then placed on ice just before use. Plasma total cholesterol, HDL-C, and triglyceride (TG) levels were determined using standard enzymatic techniques. Plasma concentrations of apoA-I, -A-II, and -B were determined by immunoturbidimetry. Plasma concentrations of apoA-IV, -C-I, -C-III, and -E were estimated by dot-blot analyses and expressed as arbitrary units. LCAT gene analyses, activity, and mass measurements were performed as described previously (14). HDL subpopulations were determined by nondenaturing two-dimensional PAGE, immunoblotting, and image analysis as described previously (15). Four microliters of plasma was applied and electrophoresed on a vertical-slab agarose gel (0.7%) in the first dimension at 250 V until the α-mobility front moved 3.5 cm from the origin. The agarose gel was sliced, and the strips were applied onto 3–35% nondenaturing concave gradient polyacrylamide gels. In the second dimension, gels were electrophoresed to completion at 250 V for 24 h at 10°C, followed by electrotransfer to nitrocellulose membranes at 30 V for 24 h at 10°C. The specific apolipoproteins were immuno-localized on the membrane with mono-specific goat anti-human primary and ^{125}I-labeled secondary antibodies [immunopurified rabbit F(ab')$_2$ fraction against goat IgG]. The bound ^{125}I-labeled secondary antibody was quantified in a FluoroImager (Molecular Dynamics). Each membrane was first probed for the apolipoprotein of primary interest and than reprobed for apoA-I for reference.

Data analysis

Means and standard deviations were calculated for all study groups. Data obtained from homozygotes and heterozygotes were compared with data from controls using ANOVA analyses. A two-tailed $P < 0.05$ was considered as significant.

RESULTS

Table 1 shows data on LCAT mass and activity as well as on lipids and apolipoproteins in controls (n = 22), heterozygotes (n = 11), and homozygotes (n = 11) for LCAT deficiency. Heterozygotes had 39% of the LCAT activity and 87% of the LCAT mass of controls. They had lower apoA-I (−22%), apoA-II (−19%), HDL-C (−15%), and

TABLE 1. Characteristics of study participants

	Controls (n = 22)	Heterozygotes (n = 11)	Homozygotes (n = 11)
Male/female	17/5	7/4	10/1
LCAT mass (μg/ml)	4.60 ± 1.01	4.02 ± 1.07	1.04 ± 0.96*
LCAT activity (nmol/ml/h)	33.0 ± 18.1	20.21 ± 1.6*	0.44 ± 0.66*
Total cholesterol (mg/dl)	200 ± 38	171 ± 37*	112 ± 63*
LDL-C (mg/dl)	126 ± 33	99 ± 33*	65 ± 54*
HDL-C (mg/dl)	54 ± 13	46 ± 12*	9 ± 5*
TG (mg/dl)	137 ± 88	127 ± 45	203 ± 146*
apoA-I (mg/dl)	140 ± 25	109 ± 17*	34 ± 11*
apoA-II (mg/dl)	38 ± 4	31 ± 5	11 ± 6*
apoB (mg/dl)	96 ± 16	97 ± 25	31 ± 17*

ApoA-I, apolipoprotein A-I; HDL-C, HDL cholesterol; TG, triglyceride. Data are mean ± SD.
* Significantly different ($P < 0.05$) from controls.

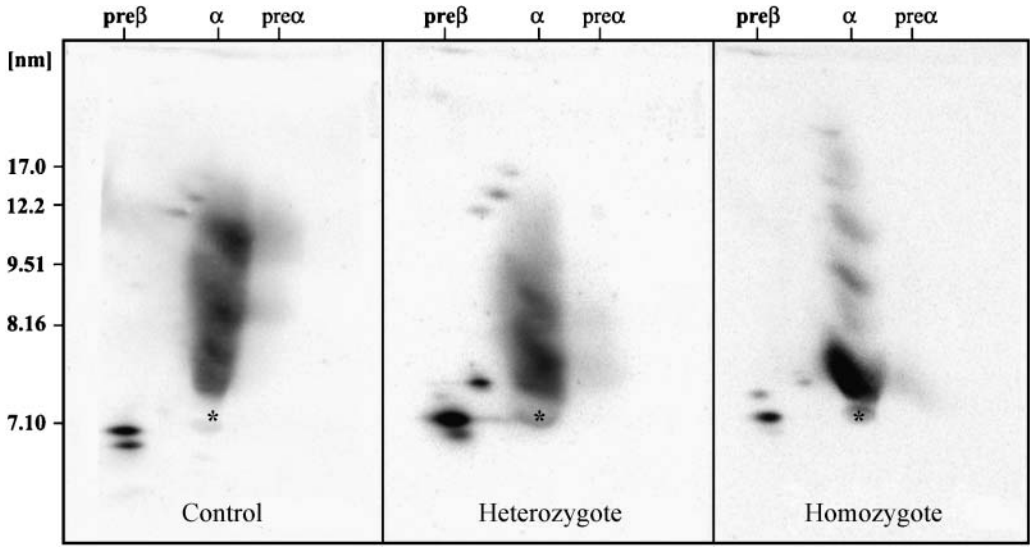

Fig. 1. Apolipoprotein A-I (apoA-I)-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects separated by two-dimensional, nondenaturing agarose-PAGE. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

TG (−7%) values compared with controls. Homozygotes had about 1% of the LCAT activity and about 23% of the LCAT mass of controls. They had significantly lower HDL-C (−83%), apoA-I (−76%), apoA-II (−71%), apoB (−68%), and LDL-C (−49%), and 48% higher TG values than controls.

Figure 1 and **Table 2** summarize data on apoA-I-containing HDL subpopulations in controls and in heterozygous and homozygous LCAT-deficient subjects. Heterozygotes had one extra particle in the preβ-1 region (preβ-1×); however, all of their other apoA-I-containing HDL subpopulations were comparable to controls in electrophoretic mobility and size. ApoA-I distribution in heterozygotes was shifted toward the smaller HDL particles: there was a 2-fold increase in preβ-1 level, a 23% increase in α-4, and a 45% increase in preα-4 levels compared with controls. There were significant decreases in the concentrations of all the other HDL particles, whereas the mean concentration of α-3, an intermediate-sized particle, was similar to that of controls. In homozygotes, the majority of apoA-I was detected in small, lipid-poor, disc-shaped HDL particles (preβ-1 and α-4). Despite the low plasma concentrations of apoA-I in homozygous subjects, the apoA-I concentrations of these particles were comparable to those of controls. We have also observed larger (~8 nm–20 nm) apoA-I-containing HDL particles with α-mobility in many of the homozygotes. **Figure 2** represents the distribution of apoA-II-containing particles—superimposed on apoA-I-containing particles—in representative control, heterozygous, and homozygous LCAT-deficient subjects. In control subjects, α-2 and α-3 HDL contain apoA-I and apoA-II. In heterozygotes, some apoA-II was detected in the preβ-1 region but the majority of apoA-II was distributed in the α-2 and α-3 subpopulations, with a slight shift toward the smaller α-3 particles, compared with controls. In contrast to controls, homozygotes had a very low level of apoA-II, which was detected in a small, lipid-poor particle, comigrating with the regular LpA-I preβ-1 HDL particles. Total or partial LCAT deficiency had no significant effect on the concentration of apoA-IV or the distribution of apoA-IV-containing HDL particles (**Fig. 3**). There were no significant differences between heterozygotes and controls in apoC-I concentration and distribution (**Fig. 4**). In contrast, homozygotes had significantly lower apoC-I levels, and their apoC-I was found on the top of the gel with β-mobility, indicating that apoC-I was present solely in VLDL particles, not in α-mobility HDL particles, as in controls and heterozygotes. The concentrations and distribution of apoC-III were significantly different between LCAT-deficient subjects and controls (**Fig. 5**). In controls, the majority of apoC-III comigrated with apoA-I in α-1 and α-2 HDL, and some was also found

TABLE 2. Concentrations of HDL subpopulations as determined by apoA-I content

	Controls (n = 22)	Heterozygotes (n = 11)	Homozygotes (n = 11)
Preβ-1_x	Not detectable	0.7 ± 0.9*	1.6 ± 1.0*
Preβ-1_a	8.2 ± 3.2	14.6 ± 5.4*	7.9 ± 4.0
Preβ-1_b	4.1 ± 1.6	10.3 ± 8.1*	1.8 ± 1.1*
Preβ-2_a	0.7 ± 0.4	0.3 ± 0.2*	Not detectable
Preβ-2_b	1.0 ± 0.5	0.5 ± 0.3*	Not detectable
Preβ-2_c	0.5 ± 0.3	0.2 ± 0.2*	Not detectable
α-1	16.7 ± 8.9	11.0 ± 8.6*	
α-2	39.1 ± 9.6	25.3 ± 6.7*	11.6 ± 2.4
α-3	24.3 ± 5.6	23.2 ± 5.5	
α-4	13.4 ± 3.6	16.5 ± 3.5*	12.1 ± 7.0
Preα-1	5.2 ± 3.3	0.9 ± 0.9*	
Preα-2	6.2 ± 2.4	1.9 ± 1.1*	0.6 ± 0.4
Preα-3	3.4 ± 1.4	2.0 ± 1.0*	
Preα-4	1.1 ± 0.4	1.6 ± 0.8*	0.6 ± 1.0

Data are mean (mg/dl) ± SD.
* Significantly different ($P < 0.05$) from control.

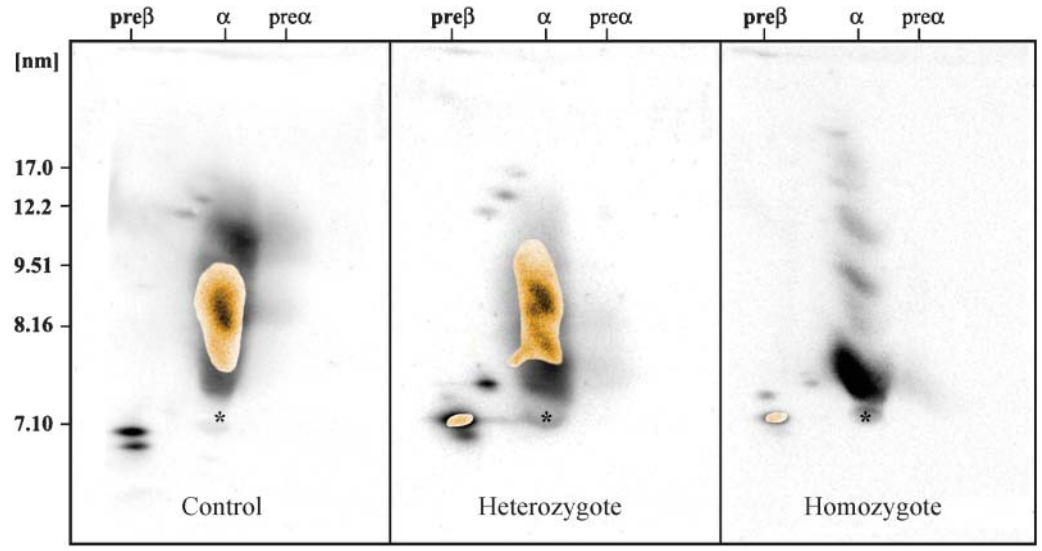

Fig. 2. ApoA-II-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects superimposed on the image of apoA-I-containing subpopulations. LCAT-deficient subjects have apoA-II in small, preβ-migrating HDL particles. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

in the α-3 and α-4 size range, with no comigration with apoA-I. In contrast, practically all of the apoC-III was detected in small, lipid-poor HDL particles in homozygotes and heterozygotes. ApoE-containing particles migrated with β-preβ-mobility in the size range between 12 nm and VLDL size, with a median diameter of 16.5 nm in controls (**Fig. 6**) and no overlap with apoA-I-containing HDL particles. In heterozygotes, apoE was also found in large β-preβ-mobility particles, with no comigration with apoA-I. Interestingly, the size of apoE-containing particles was somewhat increased in heterozygotes compared with controls. Homozygotes had much less apoE than controls. ApoE concentrations in the larger particles decreased, and smaller apoE-containing particles appeared in the plasma of homozygotes.

DISCUSSION

The purpose of this study was to gain insight into the role that LCAT plays in HDL metabolism as well as to better understand LCAT deficiency states. Characteriz-

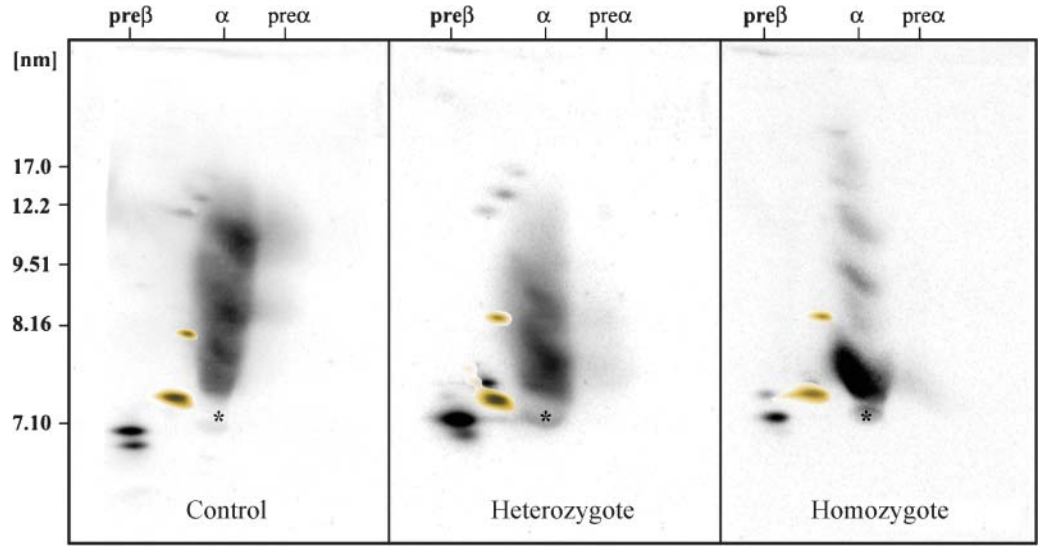

Fig. 3. ApoA-IV-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects superimposed on the image of apoA-I-containing subpopulations. Total or partial LCAT deficiency has no significant effect on the distribution of apoA-IV-containing HDL particles. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

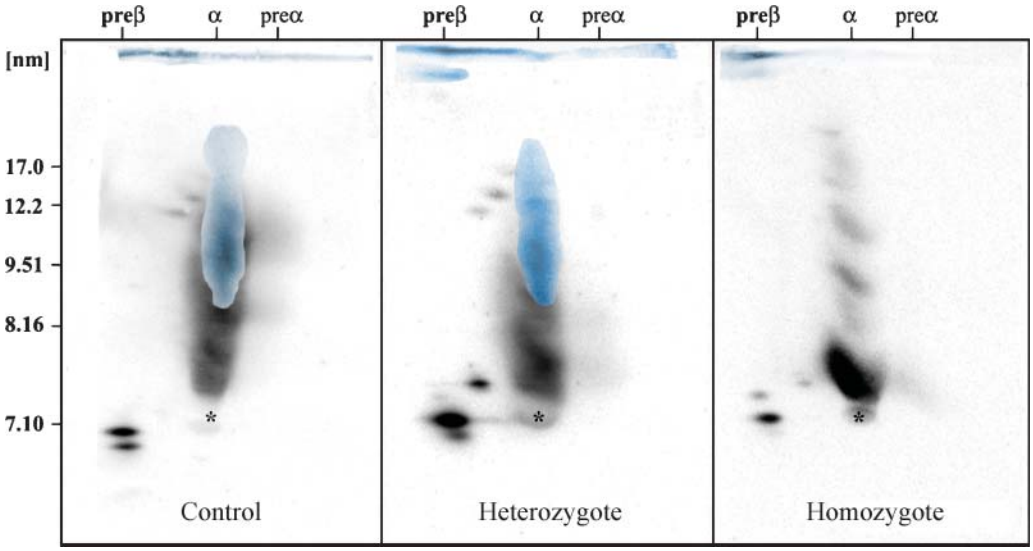

Fig. 4. ApoC-I-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects superimposed on the image of apoA-I-containing subpopulations. In homozygotes, apoC-I has only been detected on the top of the gel with β-mobility (VLDL) in contrast to controls and heterozygotes. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

ing HDL particles in patients with rare inborn errors of HDL metabolism has been helpful in better understanding HDL particle metabolism and reverse cholesterol transport. We have documented that Tangier disease patients had: *1*) apoA-I only in the preβ-1 HDL particles, *2*) no apoA-II-containing HDL, and *3*) decreased size of apoE HDL. ApoA-IV was not significantly influenced by the lack of ABCA1-mediated cellular cholesterol efflux (16). We have subsequently reported that HDL subpopulations in CETP-deficient homozygotes were very large, compositionally undifferentiated HDL particles (17). Therefore, CETP activity is essential for the formation of distinguished HDL particles in the normal size range of HDL. Most importantly, CETP activity is essential for the formation of discrete LpA-I, LpA-I:A-II, and LpE HDL particles.

In the present manuscript, we document the role of LCAT in HDL metabolism and remodeling in plasma. The first important observation is that LCAT activity is not

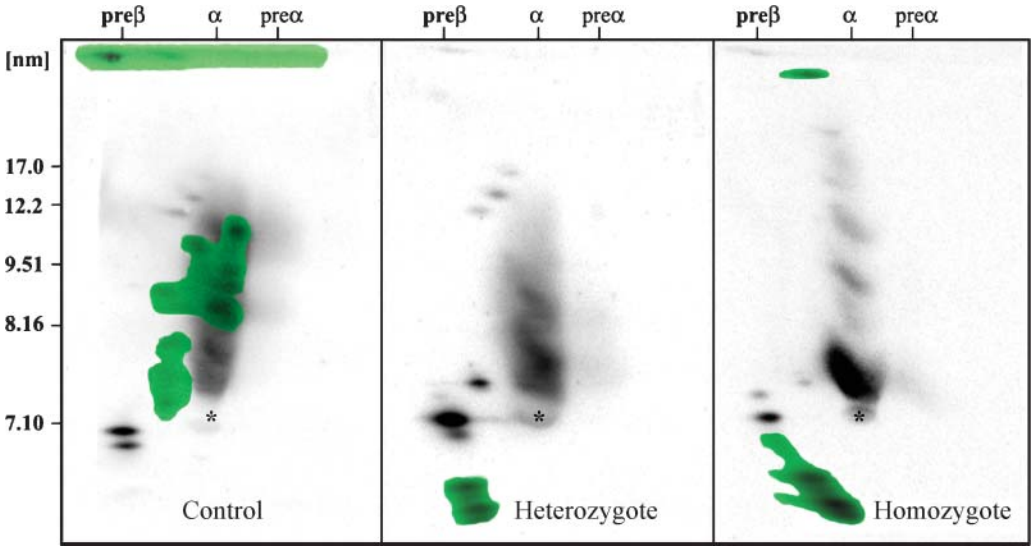

Fig. 5. ApoC-III-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects superimposed on the image of apoA-I-containing subpopulations. In controls, the majority of apoC-III comigrates with apoA-I in α-1 and α-2 HDL, and some has also been found in the α-3 and α-4 size range with no comigration with apoA-I. In homozygotes and heterozygotes, practically all apoC-III has been detected in small, lipid-poor HDL particles. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

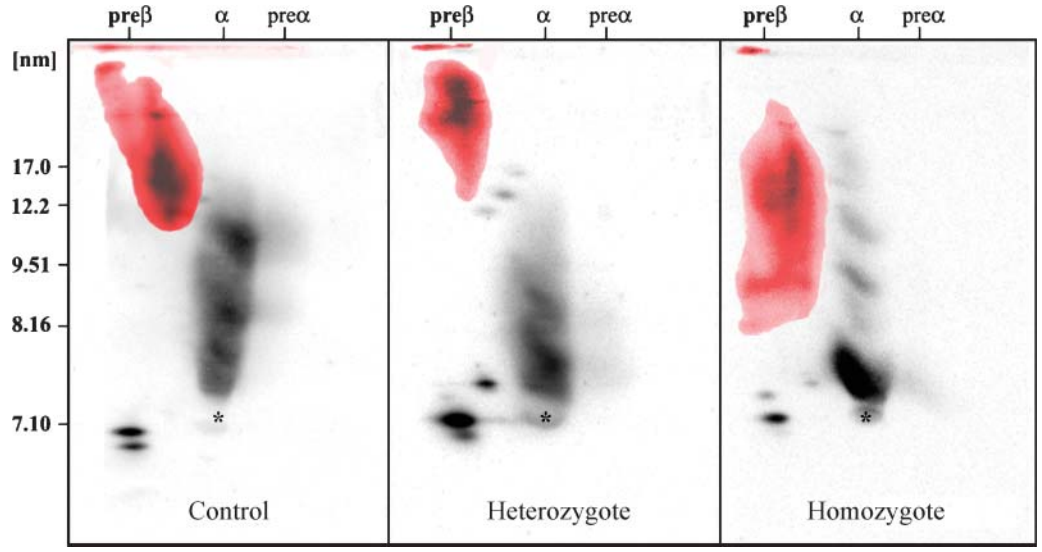

Fig. 6. ApoE-containing HDL subpopulations of representative control, heterozygous, and homozygous LCAT-deficient subjects superimposed on the image of apoA-I-containing subpopulations. There is no comigration of apoE- and apoA-I-containing particles. The asterisk represents the endogenous human serum albumin marking the α-mobility front.

necessary for the transformation of preβ-1 HDL into α-mobility HDL. Preβ-1 binds to ABCA1 and removes phospholipids and unesterified cholesterol from cells (13). During this process, there are probably changes in apoA-I conformation and electrophoretic charge. We hypothesize that α-4 HDL contains two molecules of apoA-I, as is the case for preβ-1 HDL. Larger (~8 nm–20 nm) α-mobility HDL particles have also been observed in many of the homozygotes. The tight bands of these particles suggest that these are poorly lipidated, discoidal HDL aggregates. We have no data indicating whether LCAT can act on these large, stacked disks or can use only the small α-4 HDL as a substrate. The apoA-I-containing HDL subpopulation profile of heterozygotes resembles that of low HDL-C CAD patients, inasmuch as apoA-I distribution is shifted toward the smaller particles. ApoA-II is dramatically reduced in homozygous subjects, probably because of fast catabolism (18), and, interestingly, it comigrates with preβ-1 HDLs, which normally contain only apoA-I. Some apoA-II also comigrates with preβ-1 HDL in heterozygotes; however, we do not know whether apoA-I and apoA-II are in the same particles. As a result of the presence of cholesteryl ester in the core of HDL particles, apoA-II binds to α-2 and α-3 HDL particles very early, as indicated in heterozygotes whose apoA-I/apoA-II ratios are increased in these particles. Our data also suggest that LCAT is not a key player in the formation of apoA-IV-containing particles. On the basis of these and other findings (16, 17), we hypothesize that the metabolism of apoA-IV-containing particles is independent of ABCA-1-mediated cellular cholesterol efflux, as well as of CETP and LCAT activities in humans. The majority of apoC-I comigrates with apoA-I-containing α-1 HDL in controls. About 20% of apoC-I in controls and ~35% of apoC-I in heterozygotes have α-mobility with larger than α-1 size. Homozygotes have apoC-I only in the VLDL fraction, indicating that the neutral lipid core is essential for the incorporation of apoC-I into HDL. ApoC-III has a complex pattern in controls: ~25% of apoC-III comigrates with α-1, ~50% comigrates with α-2, ~15% is found in VLDL, and the rest is in the HDL size range but does not overlap with apoA-I-containing particles. Interestingly, in both affected groups, apoC-III has been detected in small, lipid-poor form (free apoC-III), indicating that apoC-III is probably sensitive to the lipid and apolipoprotein composition of HDL. The large amount of free apoC-III in affected subjects also indicates that the fractional catabolic rate of this apolipoprotein is not increased with decreased particle size, which is clearly not the case for apoA-I and apoA-II. We clearly demonstrate that apoE-containing particles do not overlap with apoA-I-containing particles either in controls or in LCAT-deficient subjects in this study. [We have only seen apoE comigrating with apoA-I in homozygous CETP-deficient subjects where HDL size reached the size of LDL and the particles were probably loaded with excess amounts of cholesteryl ester (17)]. Although apoA-I concentrations were significantly lower in the large particles in heterozygous LCAT-deficient subjects, apoE concentrations were significantly increased in the large apoE HDL particles in these subjects. We have no explanation for this phenomenon. We do not know the chemical composition of these particles. In homozygous LCAT-deficient subjects, we observed only slightly more apoE in apparently lipid-poor particles. Therefore, LCAT activity does not seem to be a key player in supplying neutral lipids for apoE-containing HDL. Alternatively, TGs seem to be sufficient for the formation of the core of apoE HDL in homozygotes. If this is true, the questions arise as to how this TG-rich apoE HDL is metabolized and what its role in lipoprotein metabolism and CAD risk is.

Our current concept of HDL remodeling in vivo in humans, presented in **Fig. 7**, is based on data generated in various genetic states associated with alterations in HDL metabolism (ABCA1, LCAT, and CETP deficiency). We are in the process of examining HDL subpopulations in other genetic disorders as well [apoA-I deficiency, apoE deficiency, abetalipoproteinemia, lipoprotein lipase (LPL) deficiency, and hepatic lipase (HL) deficiency]. On the basis of our observations, we describe the following steps in HDL metabolism.

Step 1: synthesis

ApoA-I is synthesized in the liver and small intestine, and two molecules of apoA-I form a belted structure around ~16 molecules of phospholipids to form discoidal preβ-1 HDL in the interstitial or plasma compartment.

Step 2: cellular cholesterol efflux

Preβ-1 particles pick up FC and more phospholipids from cells via the ABCA1 pathway and are transformed into small, lipid-poor, discoidal LpA-I α-4 HDL particles.

Step 3: cholesterol esterification

LCAT esterifies FC on the surface of HDL into cholesteryl esters, which move into the core with an increase in HDL particle size.

Step 4: TG hydrolysis

LPL hydrolyzes TGs in TG-rich lipoprotein (TRL), resulting in surface components (phospholipids, FC, and apolipoproteins) available and necessary for HDL particle size increase.

The concerted actions of ABCA1, LCAT, and LPL continuously increase HDL particle size (steps 2–4).

Step 5: cholesteryl ester exchange (CETP cycle)

With CETP-mediated exchange of core cholesteryl esters for TG between large HDL particles and TRL, differentiated α- and preα-migrating HDL particles form; these contain apoA-I with apoA-II, or apoA-I without apoA-II, or apoE only. CETP can also exchange cholesteryl esters for TG among HDL particles and, as a result, a substantial amount of preβ-1 forms.

Step 6: phospholipid hydrolysis

HL, endothelial lipase (EL), and secretory phospholipase A2 (sPLA2) hydrolyze TG and phospholipids on HDL, resulting in size reduction of large α-1 into α-2 HDL (HL), or disintegration of all larger HDL particles into α-4 and preβ-1 (EL), or preβ-1 and free apoA-I (sPLA2).

Step 7: hepatic cholesteryl ester uptake (SR-BI cycle)

Cholesteryl esters on α-2 and α-1 HDL particles are selectively transported from HDL particles to the liver via SR-BI for ultimate excretion of cholesterol into the bile, resulting in recycling of apolipoproteins, phospholipids, and FC from these larger HDL particles to small α-4 HDL.

The concerted actions of CETP, SR-BI, and lipases decrease HDL particle size (steps 5–7).

Step 8: apolipoprotein catabolism

Clearance of small, lipid-poor apoA-I particles: the final step in HDL particle metabolism is the uptake of whole HDL particles by the liver and cubulin/megalin-mediated clearance of free apoA-I and preβ-1 HDL in the kidney.

Based on the observations presented here, our data are consistent with the concept that LCAT plays a crucial role in the maturation of HDL particles (steps 2–4).

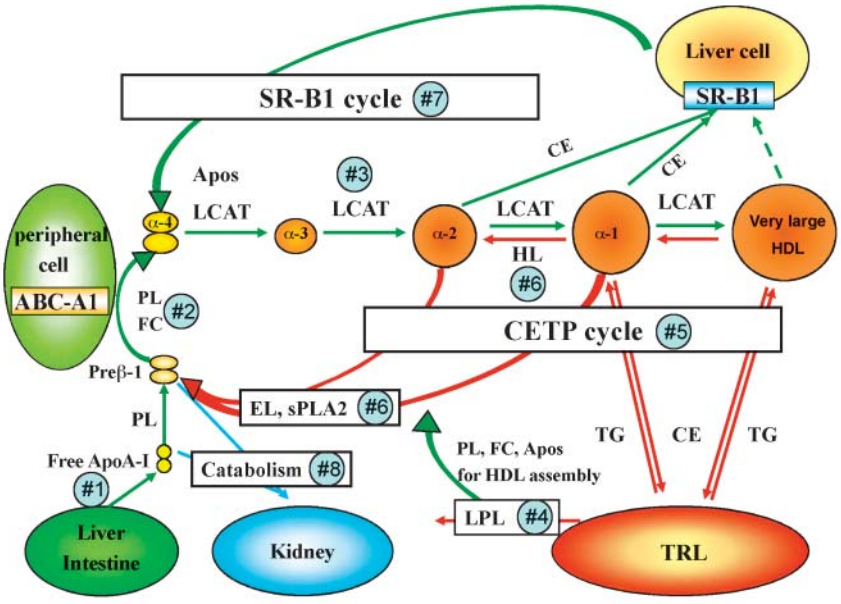

Fig. 7. Current concept of HDL remodeling in humans in vivo. Abbreviations not used in the text: phospholipids (PL), cholesteryl ester (CE).

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