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High-density lipoprotein subpopulation profiles in lipoprotein lipase and hepatic lipase deficiency

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Abstract

Background and aims—Our aim was to gain insight into the role that lipoprotein lipase (LPL) and hepatic lipase (HL) plays in HDL metabolism and to better understand LPL- and HL-deficiency states.

Methods—We examined the apolipoprotein (apo) A-I-, A-II-, A-IV-, C-I-, C-III-, and E-containing HDL subpopulation profiles, assessed by native 2-dimensional gel-electrophoresis and immunoblotting, in 6 homozygous and 11 heterozygous LPL-deficient, 6 homozygous and 4 heterozygous HL-deficient, and 50 control subjects.

Results—LPL-deficient homozygotes had marked hypertriglyceridemia and significant decreases in LDL-C, HDL-C, and apoA-I. Their apoA-I-containing HDL subpopulation profile was shifted toward small HDL particles compared to controls. HL-deficient homozygotes had moderate hypertriglyceridemia, modest increases in LDL-C and HDL-C level, but normal apoA-I concentration. HL-deficient homozygotes had a unique distribution of apoA-I-containing HDL particles. The normally apoA-I:A-II, intermediate-size (α-2 and α-3) particles were significantly decreased, while the normally apoA-I only (very large α-1, small α-4, and very small preβ-1)

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particles were significantly elevated. In contrast to control subjects, the very large α-1 particles of HL-deficient homozygotes were enriched in apoA-II. Homozygous LPL- and HL-deficient subjects also had abnormal distributions of apo C-I, C-III, and E in HDL particles. Values for all measured parameters in LPL- and HL-deficient heterozygotes were closer to values measured in controls than in homozygotes.

Conclusions—Our data are consistent with the concept that LPL is important for the maturation of small discoidal HDL particles into large spherical HDL particles, while HL is important for HDL remodeling of very large HDL particles into intermediate-size HDL particles.

Keywords

Apolipoproteins; Lipoprotein metabolism; HDL particles; HDL remodeling; Reverse cholesterol transport

Introduction

A low level of high-density lipoprotein cholesterol (HDL-C) is recognized as a major independent risk factor for the development of coronary heart disease (CHD) (1–3). HDL is a heterogeneous class of lipoprotein particles that differ in their protein and lipid composition and physiologic functions. Several enzymes play key roles in HDL remodeling *in vivo* including lipoprotein lipase (LPL) and hepatic lipase (HL).

LPL facilitates triglyceride (TG) lipolysis, while HL acts equally on TG and phospholipids. Both lipases reside mainly on the luminal surface of the capillary vascular endothelium. LPL hydrolyzes TG in triglyceride-rich lipoproteins (TRL) such as chylomicron and very-low density lipoprotein (VLDL), thus generating free fatty acids for uptake in adipose tissue as well as skeletal and cardiac muscle (4–6). TG hydrolysis by LPL reduces the core material of TRL, triggering release of surface components of these particles, namely apolipoproteins (except apoB), free cholesterol, and phospholipids, which are then transferred in part to HDL particles. It is well-documented that LPL deficiency causes very high levels of TRL and low levels of HDL-C (4–6).

HL is synthesized and secreted from hepatocytes and anchored to the liver sinusoidal surface by proteoglycans (7). HL hydrolyzes TG and phospholipids in apoB-containing lipoproteins and HDL, resulting in the formation of small HDL remnants (8). On the other hand, HL-deficient subjects have buoyant low-density lipoprotein (LDL) particles, increased cholesterol levels in HDL_2, and HDL in these subjects is also enriched in TG (9–12). Hence, HL plays a role in determining the size, density, and metabolic fate of these lipoprotein particles by modulating their lipid composition (13). Ruel *et al.* have reported that HL-deficient homozygotes had substantial amounts of TG-enriched, large-size HDL particles (14). Three of those patients are also part of our present report.

In the past, our laboratory has characterized the apoA-I content of individual HDL particles as separated by 2-dimensional gel-electrophoresis of whole plasma followed by immunoblotting and image analysis. We have noted the consistent appearance of apoA-I in ten distinct HDL particles including the very small, discoidal preβ-1 HDL (5.6 nm), small,

discoidal α-4 HDL (7.4 nm), medium-size α-3 HDL (8.1 nm), large α-2 HDL (9.2 nm), and very large α-1 HDL (11.0 nm) particles (15–19). We have also noted that apoA-II is found predominantly within medium-size α-3 and large α-2 HDL (apoA-I:A-II HDL particles), while none of the other HDL particles contain appreciable amounts of apoA-II, hence they have been denoted as apoA-I-only HDL particles (20). ApoE and apoA-IV are found on HDL particles that are each distinct from apoA-I-containing HDL particles (20). We have also documented that females have higher levels of apoA-I in very large α-1 and large α-2 HDL than males do (18). Both male and female patients with CHD, who are not on lipid-altering medications, have significantly lower levels of apoA-I in very large α-1 HDL and significantly higher levels of apoA-I in very small preβ-1 HDL than healthy gender-matched control subjects do (15–19). Furthermore, we have documented that the concentrations of apoA-I in specific HDL particles are better markers of cardiovascular disease (CVD) risk than HDL-C is and that their levels predict progression or regression of angiographically-defined coronary artery disease (16–19). We have also characterized the specific and distinct apoA-I-containing HDL particle profiles of patients with deficiencies in apoA-I, ATP-binding cassette transporter A1 (ABCA1), lecithin:cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP), due to specific molecular defects (20–23).

In this study, we have now characterized the apoA-I, apoA-II, apoA-IV, apoC-I, apoC-III, and apoE content and distribution of HDL particles in homozygous and heterozygous LPL- and HL-deficient subjects and controls in order to better understand the roles of these two lipases in HDL particle metabolism.

Materials and methods

Subjects

Plasma samples from 5 homozygous, 1 compound heterozygous, and 4 heterozygous LPL-deficient patients were obtained. The patients were 4 males and 2 females, aged 23 to 43 years, with marked hypertriglyceridemia (> 1,000 mg/dL) and absent LPL activity in post-heparin plasma. They had no other diseases and were not on any medications that affect lipoprotein metabolism. The reported amino acid substitutions in homozygotes due to mutations in the LPL gene included: 1) Ala176→Thr, 2) Gly188→Glu, 3) Ile194→Thr, 4) Ile205→Ser, 5) Pro207→Leu, and 6) a compound heterozygote with a heterozygous Gly188→Glu amino acid substitution and a nonsense mutation in exon 8 W394X (24, 25).

Plasma samples from 3 homozygous, 3 compound heterozygous, and 11 heterozygous HL-deficient patients were also obtained. HL homozygote-1 was a 56-year-old male noted to have significant hypertriglyceridemia, low post-heparin plasma LPL activity, and absent HL activity, with a homozygous intron-1 acceptor splice site mutation in the HL gene. He had sustained a myocardial infarction at age 41 years. HL homozygote-2 was a 53-year-old male with a major deletion involving the HL gene promoter and exon-1, modest hypertriglyceridemia, and absent post-heparin plasma HL activity and normal LPL activity off medications. He had sustained a myocardial infarction at age 37 years. HL homozygote-3 was a 64-year-old male with significant hypertriglyceridemia, low post-heparin LPL activity and absent HL activity, and a missense mutation Gly225→Arg in HL. He had sustained a myocardial infarction at age 42 years. All of these patients had

significant plasma TG lowering and normalized their post-heparin LPL activity on treatment with fibrate therapy (26). The three compound heterozygotes were a female and her two brothers with very low or undetectable post-heparin plasma HL activity due to a missense mutation in exon 5 Ala174→Thr and a missense mutation Thr383→Met in exon-8 of the HL gene (14). These latter patients also were noted to have moderate hypertriglyceridemia and marked TG enrichment of LDL and HDL particles.

Control subjects (25 males and 25 females) were selected from our previous studies based on having plasma samples available, no history of CVD or diabetes, and having a normal lipid profile (LDL-C < 130 mg/dl, HDL-C >40 mg/dl for males and >50 mg/dl for female, and TG <150 mg/dl).

Neither the patients nor the control subjects were on lipid-lowering medications at the time of sampling. All samples were frozen at −80 °C and never thawed until just prior to the analysis described below.

Biochemical analyses

Total-C, TG, HDL-C, and LDL-C concentrations were determined by standard enzymatic methods on an Olympus AU 400 analyzer using kits from Beckman-Coulter (Indianapolis, IN). ApoA-I concentrations were measured using a turbidimetric immunoassay (Wako Diagnostics, Richmond, VA). Within and between run coefficients of variation for all assays were < 5%. Due to hyperlipidemia, the concentrations of less abundant apolipoproteins (A-II, A-IV, C-I, C-III, and E) could not be reliably measured from plasma; therefore we assessed only the distribution/co-migration of these apolipoproteins. Distributions of apo A-I, A-II, A-IV, C-I, C-III, and E in HDL particles were determined by non-denaturing 2-dimensional gel electrophoresis (2d-ndPAGE) of plasma followed by transferring HDL on nitrocellulose membrane and immunoblotting with specific antibodies, as previously described (15, 20). Briefly: 4 microliters of plasma were applied and electrophoresed on a vertical-slab agarose gel in the 1st dimension. Gels were run in 4°C Tris-Tricine buffer 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% non-denaturing concave gradient polyacrylamide gels. In the 2nd dimension, gels were electrophoresed in 4°C Tris-Borate buffer at 250 V for 24 h, followed by electrotransfer to nitrocellulose membranes in 4°C Tris-Glycine buffer at 30 V for 24 h. The specific apolipoproteins were immuno-localized on the membrane with mono-specific goat anti-human primary antibodies against apolipoproteins A-I, A-II, A-IV, C-I, C-III, and E. ^{125}I-labeled immunopurified goat-rabbit F(ab′)2 IgG fraction (Thermo Fisher, cat#A24449) as secondary antibody was then utilized. The bound ^{125}I-labeled secondary antibody was quantified with a Storm 860 image analyzer (Molecular Dynamics, CA). Each membrane was first probed for the apolipoprotein of primary interest and then for apoA-I for reference. The sources of primary antibodies used in these experiments were: apo A-I, A-II, and A-IV antibodies prepared in our laboratory; apo C-I and C-III antibodies were provided by Dr. Alaupovic's laboratory as a gift; apoE antibody was purchased from Biodesign (cat#K74190G). All antibodies were tested and validated for specificity by SDS-gel electrophoresis of normal human plasma followed by transferring the gel to

nitrocellulose membrane and immunoblotting with the selected first antibodies and ^{125}I-labeled secondary antibody.

Results

LPL deficiency

In Table 1, we present the mean (± SD) lipid and lipoprotein concentrations of 50 control, 6 homozygous and 4 heterozygous LPL-deficient subjects. Since the measured parameters in the compound heterozygote were not different from those in the homozygotes, we present all six subjects in the homozygote group. In the general population, HDL-related parameters are significantly different between males and females; however, this gender difference was not present in LPL-deficient homozygotes, therefore, we have not separated the data by gender. Compared to controls, complete lack of LPL activity was associated with about 21-fold higher than normal mean TG level and significantly lower LDL-C (−37%), HDL-C (−75%), and apoA-I (−67%) levels. With regard to HDL particles, homozygotes had a mean increased concentration of apoA-I in preβ-1 HDL (+35%), while apoA-I in other HDL particles decreased with increasing size: small α-4 (−23%), medium α-3 (−61%), large α-2 (−74%), and very large α-1 (−89%), as compared to controls. Decreases of apoA-I in the latter three HDL particles were significant (p<0.05). The apoA-I-containing HDL subpopulation profiles of a representative control, a heterozygous, and a homozygous LPL-deficient subject are shown in Figure 1. In Supplementary Figure 1, we present the apoA-I-containing HDL subpopulations of each homozygous LPL-deficient subjects, LPL-deficient (patient 6) being the compound heterozygous subject. A shift towards smaller particles, especially preβ-1, and lack of larger α-mobility particles is visible in LPL-deficient homozygotes. The HDL subpopulation profile in LPL heterozygotes was slightly skewed toward small HDL particles, but the differences from controls did not reach significance.

The distributions of apoA-II, apoC-I, apoC-III and apoE in a representative control and a homozygous LPL-deficient subject are shown in Figure 2. The overall signal of apoA-II in homozygotes was low but the distribution and sizes of the apoA-II-containing HDL particles (α-2 and α-3) appeared to be normal. Most of apoC-I in controls was associated with very large α-1 HDL particles, while in homozygotes, only trace amount of apoC-I were seen in HDL, due to a marked decrease of its normal carrier particle, α-1 HDL. In controls, about 85% of apoC-III was found in various-sized α-mobility HDL particles with a small amount of apoC-III being found in very small, lipid-poor, preβ-migrating HDL particles. In contrast, in LPL-deficient homozygotes, most of apoC-III was found in very small, lipid-poor, preβ-migrating HDL particles. In controls, a small amount of apoE was found in VLDL and the majority was in distinct very large (> 11–20 nm diameter) LpE particles with preβ- or slow-α-mobility. Most of the LpE particles were larger than α-1 HDL. In LPL-deficient homozygotes, apoE was found in significantly smaller size HDL particles, but still with preβ- or slow-α-mobility. As in controls, these particles were separate from apoA-I-containing HDL particles. The distribution of apoA-IV in homozygotes was not different from those in controls (data not shown). No significant differences of any of the above-listed parameters were noted in LPL-deficient heterozygotes as compared to control subjects.

HL deficiency

In Table 1, we also present the mean (± SD) lipid and lipoprotein concentrations of 6 homozygous and 11 heterozygous HL-deficient subjects, compared to 50 controls. Similar to LPL-deficient patients, we have not observed significant differences between HL homozygotes and compound heterozygotes or between genders. Therefore, we again present the pulled data for complete HL-deficient patients. HL homozygotes had significantly higher plasma TG (4-fold) and HDL-C levels (+31%) than controls. They had normal plasma apoA-I level but apoA-I distribution in the HDL particles was different from normal. They had significantly increased level of apoA-I in very large α-1 (+66%), small α-4 (+65%), and very-small preβ-1 HDL particles (+215%), and significantly decreased level of apoA-I in large α-2 (−53%) and medium-size α-3 HDL particles (−44%), compared to controls. HL heterozygotes had significantly increased total and LDL-C levels, but normal levels of HDL-C, apoA-I and HDL particles.

In Figure 1, we also present the apoA-I-containing HDL subpopulation profile of a representative heterozygous and homozygous HL-deficient subject. The apoA-I-containing HDL subpopulations of the three homozygous (patients 1–3) and the three compound heterozygous (patients 4–6) HL-deficient patients are presented in Supplementary Figure 2. The electrophoretograms show increased concentrations of apoA-I in the large α-1 and small α-4, and preβ-1 HDL particles as well as decreased concentrations of apoA-I in the intermediate sized α-2 and α-3.

In Figure 3, we present the distribution of apoA-II, apoC-I, apoC-III and apoE in a representative control and a representative homozygous HL-deficient subject. In controls, the majority of apoA-II was present in α-2 and α-3 HDL particles, while in HL-deficient homozygotes, all α-mobility particles were highly enriched in apoA-II. The majority of apoC-I in controls was found in very large α-1 HDL particles, while in HL homozygotes, the majority of apoC-I was present in very small particles, co-migrating with albumin. The pattern of apoC-III-containing HDL particles was similar between controls and HL homozygotes, but the distribution of apoC-III in homozygotes was markedly shifted towards very small, lipid-poor HDL particles with α- and preβ-migration. In contrast to controls, where the majority of apoE was found in <17 nm HDL particles, the majority of apoE in homozygotes was associated with VLDL particles. The distribution of apoA-IV in HDL particles in homozygotes was not different from controls (data not shown). Similar to apoA-I, the distribution patterns of other HDL apolipoproteins (A-II, A-IV, C-I, C-III, and E) in HL-deficient heterozygotes were not significantly different from those in control subjects.

Discussion

Patients with homozygous LPL deficiency are known to have marked hypertriglyceridemia along with decreased levels of LDL-C and HDL-C and to be at increased risk of developing recurrent pancreatitis (4). Our LPL-deficient homozygotes had a mean TG level of > 2,000 mg/dL and decreased concentrations of LDL-C and HDL-C. The HDL subpopulation profile of these patients was markedly shifted toward small-size HDL particles. When chylomicrons and VLDL undergo lipolysis by LPL, the core of these particles shrinks and the surface components, including phospholipids, free cholesterol, and non-apoB apolipoproteins, are

shed and provide components for the formation of larger HDL particles. Therefore, LPL is critical not only for the lipolysis and metabolism of TG-rich lipoproteins (TRL) but also for the normal transformation of small, lipid-poor, discoidal HDL particles into larger and more complex spherical HDL particles. As a consequence of LPL-mediated decrease of TRL, CETP activity decline. CETP activity correlates with the concentration of TRL, but not with the concentration of CETP protein (27). In case of LPL deficiency and high plasma TG level, CETP enriches large HDL particles with TG. As a result, HL activity increases on the TG-enriched HDL particles. As TG is hydrolyzed by HL the core of HDL particles shrink and the extra surface material (free fatty acids, unesterified cholesterol, phospholipids, and apolipoproteins) sheds and the particles' size decrease. ApoA-II, apoC-I, and apoC-III are decreased along with apoA-I especially in the large HDL particles indicating that apoAI-containing HDL particles are essential carriers for those apolipoproteins. The significant shift of apoE-containing particles toward smaller size indicates that LPL activity is also essential for the maturation of those particles.

Our homozygous HL-deficient patients had levels of TG, LDL-C, and HDL-C that were about 4-fold, 1.3-fold, and 1.3-fold higher than control values, respectively, and all had premature CVD. Though homozygotes had significantly elevated HDL-C level, their apoA-I concentration was not different from control values, however the distribution of apoA-I within HDL particles was different and unique for this condition. The normally apoA-I-only very large α-1 particles were significantly elevated and enriched in apoA-II, while the normally apoA-I:A-II, intermediate-size, α-2 and α-3 particles were significantly decreased in HL-deficient homozygotes. In these patients, the small apoA-I-only particles, α-4 and preβ-1, were also elevated. Under normal conditions, HL works in concert with LPL and CETP and it is hard to figure out its role in HDL particle metabolism. In HL-deficient homozygotes, we were able to dissect some of the individual roles of HL on the distribution and composition of HDL particles. HL deficiency not only causes changes in HDL's lipid composition but also in the redistribution of all major apolipoproteins. Moreover, we have learned that HL activity is essential for the formation of distinct LpA-I (α-1, α-4 and preβ-1) and LpA-I:A-II (α-2 and α-3) HDL particles as well as for the formation of large, lipidated apoE-containing HDL particles.

HL is not the only player in the catabolism of large HDL particles. Recently it has been documented that endothelial lipase (EL) deficiency is also associated with increased levels of large-size HDL particles, although the HDL-particle profile was different from that observed in HL deficiency (28). HL has primarily triglyceride lipase activity and acts on all lipoprotein classes, while EL hydrolyzes phospholipids primarily on HDL. In contrast to these lipases, we have not found significantly altered HDL subpopulation profile in patients with lysosomal acid-lipase (LAL) deficiency despite having significantly decreased HDL-C levels (29). It is worth noting that so far, neither EL- nor LAL-deficiency has been found to be significantly associated with CVD risk.

Previously we have characterized the specific and distinct apoA-I-containing HDL particle profiles of patients with deficiencies in apoA-I, ABCA1, LCAT, and CETP, due to specific molecular defects (20–23). We have shown that apoA-I secretion, ABCA1-dependent cell-cholesterol efflux, and cholesterol esterification via LCAT are all critical for the formation

and maturation of small, discoidal particles into larger and increasingly spherical HDL particles. Patients with complete apoA-I deficiency had HDL-C < 5 mg/dL and no apoA-I in HDL particles (21). Patients with Tangier disease (homozygous ABCA1 deficiency) had HDL-C levels < 10 mg/dL and apoA-I only in very small, discoidal preβ-1 HDL particles (22). Homozygous LCAT-deficient patients had HDL-C levels < 15 mg/dL and only discoidal HDL particles, mainly small preβ-1 and α-4 particles, containing two apoA-I molecules and in some extent larger (8–17 nm) discs containing multiple apoA-I molecules (20). We have also shown that lipid-poor, discoidal preβ-1 particles mature into large spherical HDL particles (30) and that exposure of large HDL particles to scavenger receptor BI (SR-BI) results in the disintegration of these large particles into smaller, α-3 and α-4, HDL particles (31). Subjects with CETP deficiency had markedly increased HDL-C levels and abnormally large HDL particles, which besides apoA-I also contained apoA-II and apoE, and markedly decreased concentrations of all normal-size α-mobility (α-1, α-2, α-3, and α-4) HDL particles (23). In normal subjects, in the presence of CETP, these abnormally large HDL particles are rapidly converted into distinct LpE- and a step smaller LpA-I:A-II HDL particles. The fractional catabolic rate (FCR) of apoA-I has a strong positive association with plasma TG level and an inverse association with HDL particle size (32, 33). HL-deficient patients have mainly large TG-enriched HDL particles (14) and decreased apoA-I FCR (34).

Based on our previous and present work, we have updated our model of HDL-particle metabolism and remodeling as shown in Figure 4. HDL particle formation and maturation (green lines): 1) ApoA-I is synthesized in the liver and the small intestine and is secreted in the form of monomeric apoA-I molecules, which rapidly acquire some phospholipids in the circulation. The small amount of phospholipid triggers the monomeric apoA-I molecules to dimerize and form very small discoidal preβ-1 HDL particles (22). 2) When preβ-1 particles gain cholesterol via ABCA1, the majority of preβ-1 particles are transformed into small discoidal α-4 particles containing two molecules of apoA-I. The minority of preβ-1 particles form larger particles containing 3–5 apoA-I and only small amount of lipids, therefore these particles are large discs (apoA-I aggregates). Preβ-1 particles gain cellular cholesterol via ABCA1 and are rapidly transformed into discoidal α-mobility HDL particles containing two molecules of apoA-I (α-4) and -to a lesser extent-larger discs containing 3–5 apoA-I molecules. Under normal condition, the larger multi-apoA-I-containing discs are transient with very short half-life time; however they can be detected in LCAT-deficient subjects (20). 3) LCAT esterifies free cholesterol preferentially in larger discs. 4) The increased lipid content and the presence of cholesteryl esters in the core facilitate apoA-II binding to these particles. 5) Through the action of LPL, lipids and apolipoproteins are released from TRL particles and become available for small HDL particles to mature in size and complexity. 6) In response to changes in the core and surface components, particles fuse into abnormally large (12–17 nm), undifferentiated (LpA-I:A-II:E) HDL particles. Again, under normal conditions, the half-life time of these particles are very short; however they can be seen in CETP-deficient subjects (23).

HDL particle remodeling (brown lines): 7) CETP exchanges cholesteryl ester for TG between HDL and TRL particles and transforms LpA-I:A-II:E HDL into distinct LpA-I:A-II and LpE HDL particles. 8) HL hydrolyzes TG and as the core shrinks the very large LpA-

I:A-II HDLs split into particles containing only apoA-I (small preβ-1 and large α-1 HDL) and particles containing both apoA-I and apoA-II (medium-size α-3). 9) The cholesterol-rich α-1 and α-2 HDL particles interact with SR-BI, which selectively takes up cholesterol from these particles. The remaining cholesterol-depleted particles return into the circulation as α-3 and α-4 particles (31). The end products of HDL remodeling and degradation are small α-4 and very small preβ-1 HDL particles, which are either reutilized to form larger HDL particles or catabolized: 10) Preβ-1 HDL particles are removed from plasma by the kidney and α-4 HDL by the liver (blue lines).

HDL-particle formation has recently been described based on stable-isotope-labeled apoA-I kinetics in HDL particles (35). Mendivil *et al.* suggest that multiple-size complex HDL particles are simultaneously secreted from cells and there is no precursor-product relationship between preβ-1 and larger HDL particles. In contrast, we believe that cells secrete monomer apoA-I and that spontaneously picks up some phospholipid and forms preβ-1 (a poorly-lipidated apoA-I dimer) in plasma. Multiple apoA-I-containing (multiple size) HDL discs form after preβ-1 HDL particles efflux lipids from cells (step 2). This concept is supported by Dr. Phillips' data showing that larger multiple apoA-I-containing discs form from lipid-free apoA-I and the size of the discs depends on the apoA-I/cholesterol ratio (36, 37). Our model of HDL particle metabolism is also in line with Dr. Zannis' model, which indicates that preβ-1 is the precursor of larger HDL particles (38). Moreover, we have previously reported that when partially delipidated monkey HDL (containing about 60% of apoA-I in small preβ- and α-mobility particles) was re-infused into the same animals small HDL particles were converted entirely into larger α-HDL particles indicating that small HDL particles were precursors of larger HDL particles (30).

Normally, HDL remodeling is a rapid process and many of the intermediate particles have very short half-life time. However, we can observe and measure these transient particles in patients with errors in the process of HDL metabolism/remodeling, which substantially slow the conversion of these particles. Therefore, we believe that the data we have generated on the metabolism/remodeling of HDL particles in patients with inborn errors in the process are important to complement data generated by stable-isotope labeled apoA-I in normal people.

Complete deficiency of LPL or HL results in very distinct HDL subpopulation profiles. Our data are consistent with the concept that LPL is important for the maturation of small discoidal HDL particles into large spherical HDL particles, while HL is important for HDL remodeling of very large HDL particles into intermediate-size HDL particles. These data also support our hypothesis that HDL metabolism is not a simple linear size-expansion of HDL particles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

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ABCA1	ATP-binding cassette transporter A1
apo	apolipoprotein
C	cholesterol
CE	cholesteryl ester
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CVD	cardiovascular disease
FC	free cholesterol
HL	hepatic lipase
HDL	high-density lipoprotein
LCAT	lecithin:cholesterol acyltransferase
LDL	low-density lipoprotein
Lp	lipoprotein
LPL	lipoprotein lipase
PL	phospholipid
RLP	remnant-like particle
SR-BI	scavenger receptor BI
TG	triglyceride
TRL	triglyceride-rich lipoprotein
VLDL	very-low-density lipoprotein

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1. Lipoprotein lipase (LPL) is necessary for the maturation of small lipid-poor high-density lipoprotein (HDL) into larger particles.

2. Hepatic lipase (HL) helps remodeling large HDL particles into smaller less complex particles.

3. HDL metabolism is not a simple linear size-expansion of HDL particles.
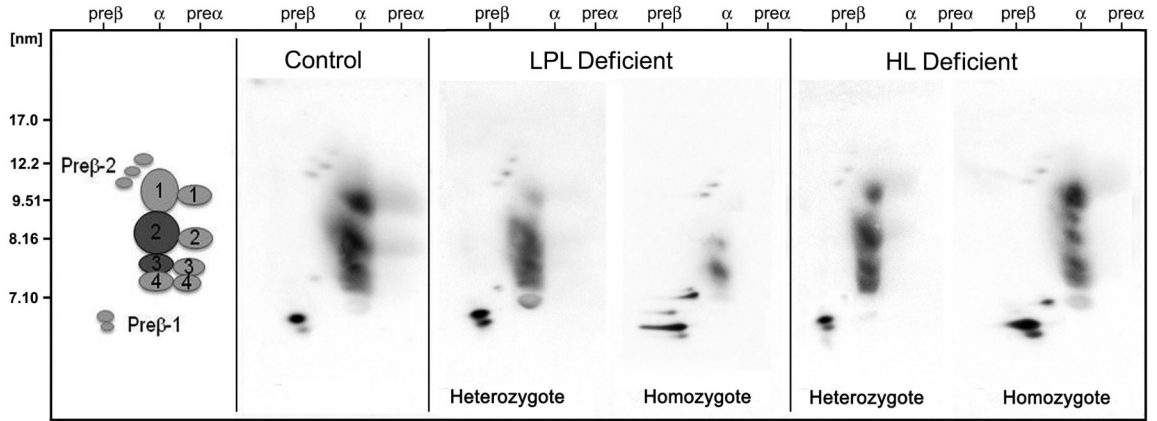
Fig. 1. ApoA-I-containing HDL subpopulations of a representative control (LDL-C=105 mg/dL, TG 87 mg/dL, HDL-C 54 mg/dL, apoA-I 165 mg/dL, and no history of CVD), a heterozygous LPL-deficient (LDL-C=120 mg/dL, TG 137 mg/dL, HDL-C 40 mg/dL, apoA-I 145 mg/dL), a homozygous LPL-deficient (LDL-C=65 mg/dL, TG 2740 mg/dL, HDL-C 14 mg/dL, apoA-I 55 mg/dL), a heterozygous HL-deficient (LDL-C=140 mg/dL, TG 120 mg/dL, HDL-C 74 mg/dL, apoA-I 175 mg/dL), and a homozygous HL-deficient (LDL-C=122 mg/dL, TG 500 mg/dL, HDL-C 64 mg/dL, apoA-I 161 mg/dL) subject

HDL particles were separated from plasma by non-denaturing 2d gel electrophoresis followed by electrotransfer of the gel content to nitrocellulose membrane and immunoprobe for apoA-I.

The first panel is a schematic representation of HDL particles: lighter grey indicates LpA-I HDL particles (containing apoA-I without apoA-II) and darker gray indicates LpA-I:A-II HDL particles (containing both apoA-I and apoA-II) in normolipidemic control subjects.
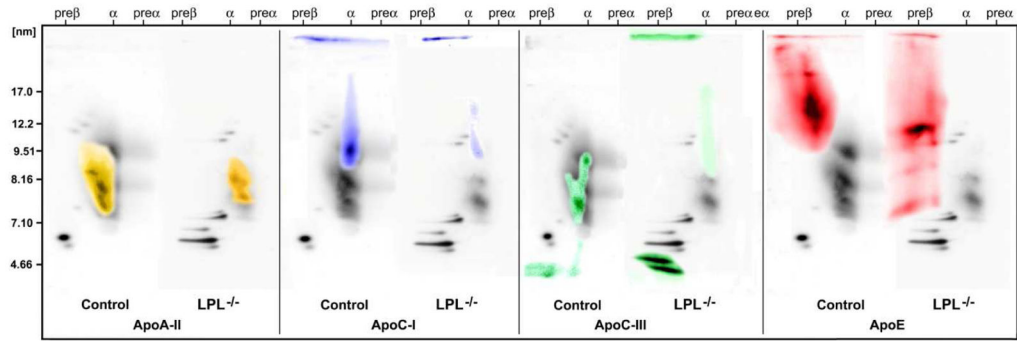
Fig. 2. ApoA-II-, apoC-I-, apoC-III-, and apoE-containing HDL subpopulations of a representative control and a homozygous LPL-deficient subject ($LPL^{-/-}$)
HDL particles were separated from plasma by non-denaturing 2d gel electrophoresis followed by electrotransfer of the gel content to nitrocellulose membrane and immunoblot for the apolipoprotein of interest. ApoA-II- (yellow), apoC-I- (blue), apoC-III- (green), and E (red) -containing HDL subpopulations are superimposed on apoA-I-containing HDL subpopulation distribution (grey). Membranes were first immunoprobed for the minor apolipoproteins and after documenting their distribution pattern, the membranes were immunoprobed for apoA-I for documenting co-migration of these apolipoproteins.
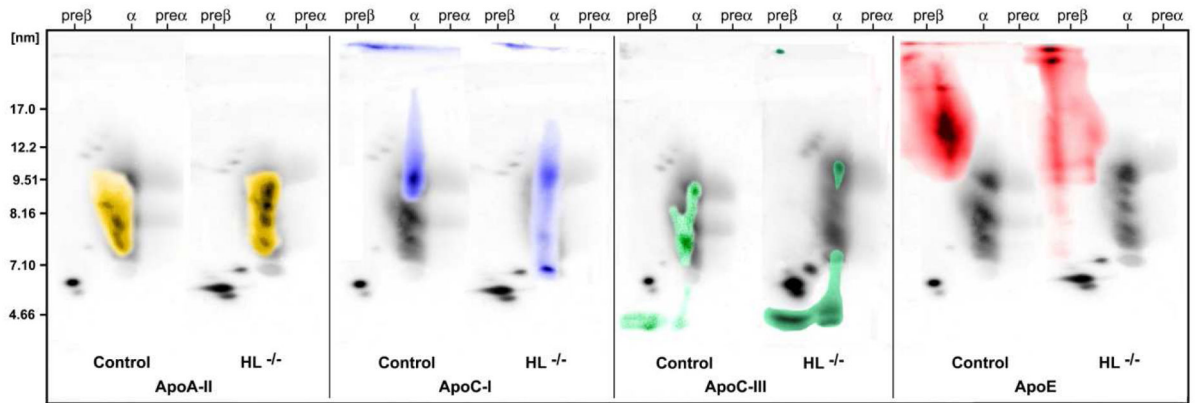
Fig. 3. ApoA-II-, apoC-I-, apoC-III-, and apoE-containing HDL subpopulations of a representative control and a homozygous HL-deficient subject ($HL^{-/-}$)

HDL particles were separated from plasma by non-denaturing 2d gel electrophoresis followed by electrotransfer of the gel content to nitrocellulose membrane and immunoblot for the apolipoprotein of interest. ApoA-II- (yellow), apoC-I- (blue), apoC-III- (green), and E (red) -containing HDL subpopulations are superimposed on apoA-I-containing HDL subpopulation distribution (grey). Membranes were first immunoprobed for the minor apolipoproteins and after documenting their distribution pattern, the membranes were immunoprobed for apoA-I for documenting co-migration of these apolipoproteins.
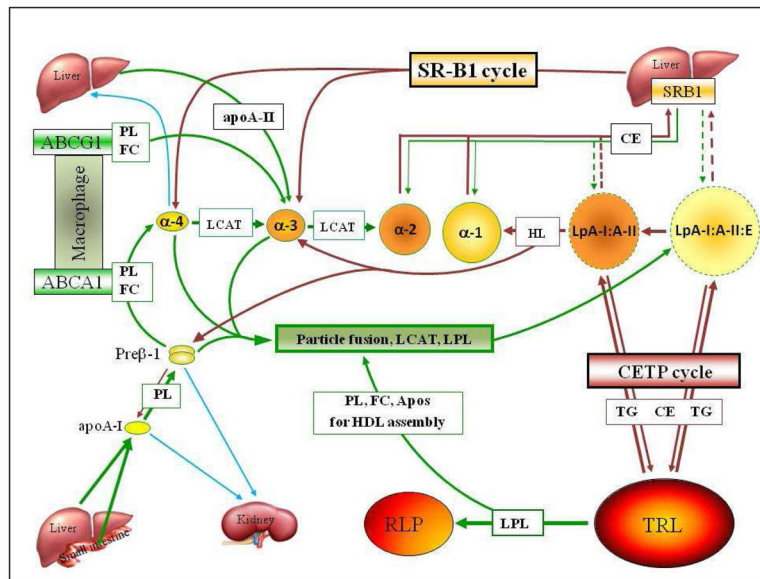
Fig. 4. A hypothetical model of the metabolism of apoA-I-containing HDL subpopulations
Green lines represent steps associated with particle maturation from small lipid-poor discs
into larger more complex spherical HDL particles. Brown lines represent steps associated
with HDL particle remodeling from larger into smaller particles. Blue lines represent steps
associated with HDL particle catabolism. ABCA1, ATP-binding cassette transporter A1;
apo, apolipoprotein; CE, cholesteryl esther; FC, free cholesterol; HL, hepatic lipase; LCAT,
lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; PL, phospholipid; RLP,
remnant-like particle; SR-BI, scavenger receptor BI; TG, triglyceride; TRL. triglyceride-rich
lipoproteins.

Table 1

Lipoprotein parameters of study participants.

	Control	LPL-deficient		HL-deficient	
		Homozygote	Heterozygote	Homozygote	Heterozygote
	(n=50)	(n=6)	(n=4)	(n=6)	(n=11)
TC	178 ± 27	311 ± 72 *	175 ± 14	265 ± 41 *	212 ± 30 *
LDL-C	99 ± 21	62 ± 21 *	125 ± 9	135 ± 37	155 ± 17 *
TG	111 ± 36	2330 ± 488 *	145 ± 24	461 ± 256 *	121 ± 31
HDL-C	59 ± 10	15 ± 8 *	45 ± 11	77 ± 14 *	65 ± 12
ApoA-I	170 ± 22	56 ± 9 *	152 ± 12	175 ± 18	165 ± 11
Major HDL particles (expressed as mg apoA-I)					
Preβ-1	8.4 ± 3.2	11.3 ± 9.5	10.1 ± 3.0	18.1 ± 6.1 *	9.1 ± 3.7
α-1	35.7 ± 13.1	3.8 ± 1.6 *	27.8 ± 8.8	59.3 ± 13.5 *	34.6 ± 10.3
α-2	66.1 ± 11.6	17.3 ± 3.5 *	61.0 ± 12.2	31.0 ± 8.1 *	65.9 ± 9.9
α-3	21.5 ± 4.0	8.3 ± 1.9 *	20.1 ± 3.9	12.1 ± 3.7 *	23.1 ± 4.5
α-4	14.7 ± 3.9	11.3 ± 1.5	16.7 ± 3.6	24.2 ± 5.1 *	16.6 ± 3.9

Numbers represent mean (mg/dL) ± SD;

*
$p<0.05$ compared to control subjects.

1mg/dL cholesterol =0.02586 mmol/L; 1mg/dL TG =0.01129 mmol/L.