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

Mariko Tani [a], Katalin V. Horvath [a], Benoit Lamarche [b], Patrick Couture [b], John R. Burnett [c], Ernst J. Schaefer [a], Bela F. Asztalos [a, *]

[a] Cardiovascular Nutrition Laboratory, Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA
[b] Institute on Nutraceuticals and Functional Foods, Laval University, Québec, Canada
[c] Department of Clinical Biochemistry, Path West Laboratory Medicine, Royal Perth and Fiona Stanley Hospital Network and School of Medicine and Pharmacology, University of Western Australia, Perth, Australia

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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) 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.
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1. 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-

Abbreviations: 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; LPL, 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.

* Corresponding author. Cardiovascular Nutrition Laboratory, Jean Mayer USDA Human Nutrition, Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA.
E-mail address: bela.asztalos@tufts.edu (B.F. Asztalos).

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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.

2. Materials and methods

2.1. 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 23–43 years, with marked hypertriglyceridemia (>1000 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.

2.2. 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 μL 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.

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