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Speciated High-Density Lipoprotein Biogenesis and Functionality

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Abstract Plasma high-density lipoprotein cholesterol (HDL-C) concentration is a negative risk factor for atherosclerotic cardiovascular disease (CVD). Despite this, most attempts to raise plasma HDL-C concentrations in a cardioprotective way have failed. Recently, hypotheses about the atheroprotective effects of HDL have shifted away from quantity to quality, mostly HDL function in reverse cholesterol transport. Plasma HDL from CVD patients is a poorer acceptor of cellular cholesterol than plasma from healthy controls, independent of plasma HDL-C concentrations. The function of HDL is likely determined by two other factors, stability and composition. The kinetic instability of HDL, which varies according to subclass, is a likely determinant of its reactivity in response to many HDL-modifying activities. HDL composition is also heterogeneous and variable; all HDL particles contain apo AI but only about two-thirds contain apo AII. This occurs despite the fact that apo AI and apo AII are hepatically secreted on separate HDL that later fuse in plasma. HDL also contains traces of other proteins, some of which have not yet been associated with HDL function. One minor HDL species are those that are secreted with intact signal peptides, which enhances their binding to HDL; these HDL have special properties that are independent of cholesterol transport. Here, we review and provide a perspective about what is currently known about speciated HDL biogenesis in the context of health and disease.

Keywords HDL · Atherosclerosis · Apolipoproteins · Cholesterol · Signal peptide

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Introduction

Plasma high-density lipoprotein-cholesterol (HDL) concentration negatively correlates with atherosclerotic cardiovascular disease (CVD) [1–3]. HDL was extensively characterized long before more recent functional characterization and is now known to have many other broad salutary properties that are determined by its different forms. HDL is highly speciated, i.e., heterogeneous with respect to density, size, and the lipid and protein compositions of various fractions. This structural and compositional heterogeneity is expected to confer functional heterogeneity on various HDL subfractions. Total HDL is comprised of a central core of neutral lipids—cholesteryl esters (CE) and triglycerides—which are surrounded by a surface of free cholesterol (FC), polar lipids—mainly phospholipids—and specialized proteins called apolipoproteins (apos). HDL occurs as an early or nascent form that is discoidal and a mature spherical form; the former is emulated by reconstituted (r) HDL formed by the in vitro association of lipids with HDL proteins.

HDL Stability Numerous studies have shown that HDL is an unstable particle and that when subjected to various physicochemical or physiological perturbations can disproportionate into two or more of its components. In her seminal paper, Gursky used differential scanning calorimetry and chaotropic perturbation to show that HDL resides in a kinetic trap; when

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perturbed, HDL escape from the trap in two irreversible denaturation steps, one on a time scale of 0.5 h due to particle rupture and release of neutral lipids which fuse in a second step on a time scale of ~5 h [4•]. Disruption of HDL by detergent and chaotropic perturbation also releases its major protein, apo AI, into the aqueous phase as a lipid-free species with a free energy of activation of 95 kJ [5, 6]; release of lipid-free apo AI is a hallmark of these reactions and HDL instability. HDL is also disrupted by several physiologically relevant activities in plasma that include non-enzymatic, lipid transfer by cholesteryl ester transfer protein (CETP) and [7] phospholipid transfer protein [8], enzymatic transformations by lecithin:cholesterol acyltransferase [9], and hepatic lipase [10]. The most profound disruption of HDL is caused by streptococcal serum opacity factor, which releases nearly half of HDL-apo AI in the lipid-free form with a free energy of activation of 95 kJ, [11•] similar to that for apo AI exchange between HDL [12]. In spite of the large amount of serum opacity factor-mediated release of apo AI, little apo AI is detected in plasma in vivo, suggesting that it rapidly reenters the HDL pool or is renally extracted [13]. Apo AI release from HDL appears to be a universal component of its interaction with transfer factors, lipolytic enzymes, and receptors, although the latter have not been tested directly.

HDL Apolipoproteins Most HDL apos are derived from the gene family of soluble apos that vary according to a primary structure and in vivo function as follows: Apo AI (28 kDa), the major HDL apo, activates lecithin:cholesterol acyltransferase (LCAT), the enzyme that transfers the SN-2 acyl chain of phosphatidylcholine (PC) to FC giving CE. On a macromolecule scale, LCAT converts discoidal HDL to spherical HDL [14], a process that converts FC to CE, a nonpolar molecule, which does not transfer between HDL and other lipoproteins in the absence of CETP. Apo AII (17 kDa) functions are still debated [15]; however, one study showed atheroprotection in rabbits [16]. During fasting, the C-peptides—apos CI, CII, and CIII—associate with HDL, but during the postprandial period transfer to intestinally derived triglyceride-rich lipoproteins, the chylomicrons. This transfer provides chylomicrons with apo CII, the activator of lipoprotein lipase, which hydrolyzes the chylomicron triglycerides (TG). Apo CI is a minor LCAT activator, and apo CIII is associated with elevated plasma TG and CVD. Apo E is an important HDL component that facilitates the removal of HDL from plasma much faster than apo E-free HDL. Apo D, which mostly occurs on HDL, belongs to the lipocalin family of proteins that binds small hydrophobic molecules [17].

In addition to well-known "apos," HDL contains many other minor proteins, although it is the smallest and most compact plasma lipoprotein (Reviewed) [18]. According to the HDL Proteome Watch Website (http://homepages.uc.edu/~davidswm/HDLproteome.html), among some 17 different proteomic analyses, 95 separate proteins have been found to associate with human HDL by at least three independent laboratories. Surprisingly, only about a third of these HDL proteins fall within the general area of lipid metabolism. The others have known functions that lie outside traditional notions of HDL function. They include proteins involved in hemostasis such as fibrinogen and several of the serine protease inhibitors (SERPINs) involved in the clotting cascade. There are also a striking number of HDL proteins involved in the inflammatory/immune response including numerous members of the complement system and its associated proteolysis inhibitors, apo J and vitronectin. Also clearly represented are both positive and negative acute-phase response proteins such as serum amyloid A and LPS-binding protein. Interestingly, there are also proteins involved in heme and iron metabolism such as hemoglobin, transferrin, and hemopexin, as well as those with a host of additional and enigmatic functions ranging from platelet regulation to vitamin binding and transport. The presence of this huge variety of proteins indicates that HDL is a functional nexus for many important biological processes that go well beyond lipid transport. Although it is outside the scope of this review to delve deeper into this topic, there are several recent reviews exploring this area [18, 19].

HDL Speciation The compositions, structures, properties, and functions of mature HDL subspecies are determined, in part, by the respective compositions, structures, and properties of the nascent HDL particles from which they are derived. HDL composition is determined by an itinerary of intracellular assembly and remodeling activities and post secretion remodeling by a milieu of lipoproteins, enzymes, and transfer proteins. Within this itinerary, post secretory HDL species contribute to HDL function through their continuous remodeling; HDL are acceptors for cholesterol efflux, LCAT substrates, and CE donors to VLDL in exchange for TG by CETP and targets of selective hepatic uptake of CE, TG, and some PLs via Scavenger receptor class B member 1 (SR-BI).

About a third of HDL particles, which occur with apo AI but not apo AII, are called LpAI. LpAI comprises particles with Stokes diameters of 10.8 and 8.5 nm. HDL with both apo AI and apo AII, called LpAI/AII, contain apo AI and apo AII in a respective 2:1 molar ratio [20], with Stokes diameters of 9.6, 8.9, and 8.0 nm. Apos C, D, and E, and LCAT occur on HDL subspecies. Whereas there are more apo AI molecules on the large than on the small HDL, LpAI/AII particles contained only two proximal apo AI molecules per particle, while the number of apo AII molecules increases from one to two and then to three with an increasing size; LpAI also comprises particles with one or two molecules of apo C-III whereas LpAI/AII particles contain ≤1 apo C-III/particle [21].

LpAI and LpAI/AII are also metabolically heterogeneous. Plasma apo AI and apo AII levels are differentially regulated.

Apo AI levels are determined primarily by catabolism [22–24] and perhaps also by production [25, 26], whereas those of apo AII are solely a function of production [22–24, 27]. The plasma residence time of apo AI on LpAI is shorter than that of apoAI on LpAI/AII (4.4 vs. 5.2 days), indicating a slower catabolism of apo AI on LpAI, AII [28]. Cholesteryl ester transfer protein interacts preferentially with LpAI [29], whereas TG and phosphatidylcholine lipolysis of postprandial LpAI/AII-HDL_2 by hepatic lipase is faster than that of postprandial LpAI-HDL_2, a finding that was supported by the increased hepatic lipolysis of LpAI by the addition of apo AII [30]. Some but not all studies suggest that LpAI is more antiatherogenic than LpAI/AII [31–33]. Although HDL also contains apo E and the C-apolipoproteins, interpretation of their plasma kinetics is made more complicated by their occurrence in the apolipoprotein B-containing lipoproteins and their redistribution during cycles from the fasted to the postprandial state in which the latter apolipoproteins transfer to chylomicrons. Apo AI likely transfers among lipoproteins, but the transfers are metabolically silent because they are between HDL and not between HDL and the B-containing lipoproteins. In contrast, apo AII appears to be nontransferable because many of the physicochemical and physiological perturbations that displace apo AI from HDL fail to do the same to apo AII [4•, 5, 11•]. Moreover, the free energy of association of monomeric apo AII with lipids was measurable [34], whereas that for dimeric apo AII was not because of its higher affinity—theoretically twice as high.

Beyond the two most common proteins apo AI and apo AII, other HDL proteins also segregate into compositionally stable particles. Asztalos et al. have shown many distinctive HDL protein patterns using two-dimensional gel electrophoresis [35]. They showed clear overlap between apoAI and apoAII, confirming the presence of LpAI/AII particles mentioned above, but they also found that other apolipoproteins migrated to distinct locations. For example, apo AIV and apo E appeared in spots that did not show significant overlap with apo AI, suggesting that they are on distinct particles. In addition, the Davidson laboratory has performed extensive proteomic profiling of HDL particles that were separated based on density [36], size [37], and charge [38]. These studies clearly indicated that HDL proteins distribute in distinct patterns across the HDL spectrum. A comigration analysis of this data revealed that certain pairs of proteins appear to migrate together across orthogonal separations, strongly suggesting that those proteins reside on the same particles [38].

The colocalization of these proteins into HDL subspecies likely has profound impact on the functionality of these particles. The best known example of functional on-particle cooperation between HDL resident proteins is the relationship of apo AI and LCAT. On its own, LCAT is relatively inefficient in mediating cholesterol esterification in lipoproteins. However, apo AI is a cofactor that stimulates this activity by several orders of magnitude when both are present on the same particle [39]. Apo F, also known as lipid transport inhibitor protein (LTIP), can inhibit the CETP-mediated exchange of CE between HDL and LDL, possibly by modulating CETP's affinity for the HDL particle surface [40]. But the most striking example of on-particle cooperation is the discovery that a specific HDL subparticle can mediate the lysis of *T. brucei* (*Tbb*), a trypanosome responsible for African sleeping sickness [41]. This HDL particle, called trypanosome lytic factor (TLF) contains apo AI, apo LI, and haptoglobin-related protein (HRP) forming a complex that is taken up by the trypanosome via the HRP moiety, allowing apo LI to permeabilize its lysosomes to lethal effect (reviewed in [42]). This is the strongest evidence yet for distinct particles within classically defined HDL that perform highly specialized functions through cooperative protein interaction. Given the multitude of known HDL proteins, it is easy to imagine the existence of additional unknown subspecies that may contribute to HDL cardioprotection and other salutary activities.

Speciated HDL Biogenesis Although nascent HDL can form via the interaction of apo AI and other exchangeable apos with macrophage ABCA1, according to studies in mice that are liver-specific apo AI-null, most plasma HDL is hepatically derived [43]. Several laboratories have characterized HDL production as follows [44–46]: Hepatocytes are the main site of apo AI and ATP-binding cassette transporter A1 (ABCA1) expression. Apo AI phospholipidation is profound and most apparent in endoplasmic reticulum and medial Golgi, both in the lumen and on the membrane fractions of the ER and medial Golgi; about half of hepatically synthesized apo AI is phospholipidated intracellularly, whereas the remainder is secreted lipid-free after which it acquires lipid. Early apo AI lipidation in ER is ABCA1-independent whereas its lipidation in Golgi and at the plasma membrane requires ABCA1. The bulk of lipidation by phospholipids and cholesterol occurs in the Golgi and at the plasma membrane, respectively.

The biogeneses of HDL containing apo AII and apo E are distinct from each other and that of apo AI. Human apo AII differs from that of other species by the occurrence of Cys6, which forms disulfide-linked homodimers and heterodimers with apos D and E [47, 48]. Although nearly all apo AII occurs as homodimers, their formation would seem unlikely. Given that the dimerization rate is the product of the monomer concentration, at [apo AII] ~400 mg/L~5×10^{-5} M, the reaction rate was predicted to be slow [47]. This hypothesis was supported by kinetic studies of apo AII that revealed that the halftime for dimerization was ~10 days, not likely a physiologically relevant lifetime given that the plasma lifetime of apo AII is on the order of 4 days, and in media from human hepatoma cell cultures only dimeric apo AII is found. However, in the presence of lipid, the rate was accelerated by a factor of ~10,000 [49]. This finding suggested that

human apo AII might be dimeric even before hepatic secretion. A pulse-chase test of this hypothesis in hepatoma cells revealed no detectable monomeric apo AII. Moreover, this same test showed that intracellular apos AI, AII, and E are independently distributed among intracellular and newly secreted particles of human hepatoma cells [44]. Only after secretion do the apos AI and AII occur on the same HDL, and apo E is transferred from VLDL to HDL; the former reaction is likely catalyzed by LCAT [50]. Thus, hepatic biogenesis of HDL-apos AI, AII, and E are distinct (Fig. 1) [44]. All homo- and heterodimeric apo AII and apo E are lipidated; monomeric apo AII and apo E are lipid-free (LF). LpAI/AII formation is a post secretory, LCAT-dependent process [50]. In HepG2 cell culture, apo dimerization rates are $(\text{apo AII})_2 > (\text{apo E})_2 > (\text{apo AII-apo E})$; thus, intracellular dimerization and lipidation are fast and concerted. No intracellular apo AII monomers were detected. Therefore, a firm conclusion is that Apo AII lipidation drives its intracellular dimerization.

HDL Secreted with Intact Signal Peptides In addition to the exchangeable apos, HDL comprises distinct particles containing unique protein complements that could be the basis of their functional diversity. Several proteomics studies reveal that HDL contains numerous minor proteins specific to certain subfractions [36, 51]. Most secreted proteins, including HDL-apolipoproteins, are synthesized with a signal peptide on the N-terminus that is cleaved prior to secretion; importantly, secretion efficiency is a function of signal peptide (SP) structure [52]. However, some proteins are secreted with intact SPs, including some HDL-apolipoproteins. Currently, these include apo L1 and haptoglobin-related protein (Hpr), components of trypanosome lytic factor (TLF), paraoxanase-1 (PON1), which is thought to atheroprotect via lipid hydroperoxide hydrolysis, and apo M, a carrier of sphingosine-1-phosphate (S1P).

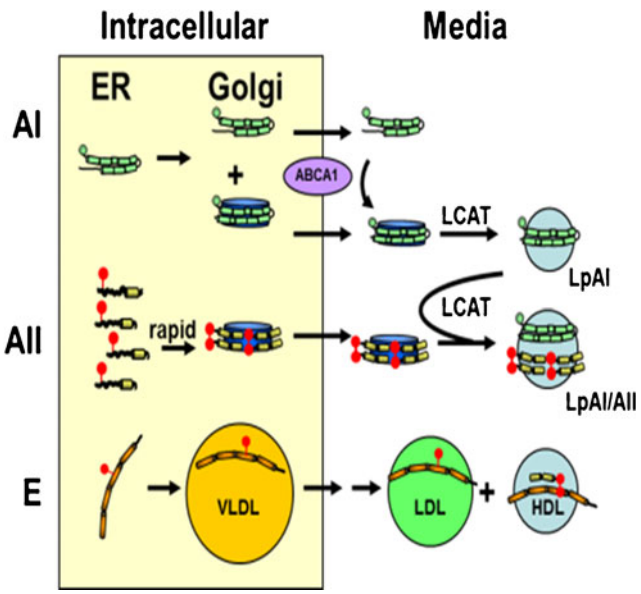

Fig. 1 Independent assembly, secretion, and remodeling of HepG2-cell-derived HDL with apos AI, AII, and E. (Reprinted from Gillard et al. with permission from Elsevier [44])

Signal Peptide Apos (SP_{apos}), a Unique Class of HDL Apos Special apos occur on HDL with intact SPs, which are lipophilic. These are apo M, apo L1, Hpr, and PON1 [53–56, 57••]; collectively, we will refer to the circulating HDL containing apos with intact SPs as HDL_{SPapos} and individually as HDL_{Hpr}, HDL_{L1}, HDL_{M}, and HDL_{PON1}. Intact SPs would be expected to impart these proteins and the HDL with which they are associated with unique properties. One of these is likely lipophilicity. The signal peptide is very hydrophobic, and the addition of such a moiety to a protein that already binds to lipids is likely to increase its lipophilicity, perhaps to the point that these proteins, like apo B-100, are nontransferable or at least only slowly transferable. Given the similar effects of increased lipophilicity of these proteins, they are likely to have similar if not common biogenic assembly and secretory itineraries.

- Apo M, a ~21 kDa protein from the lipocalin family [58] and secreted by HepG2 cells [59], contains a SP because it lacks a SP peptidase cleavage site [60]. Plasma apo M (0.9 μM) occurs on >5 % of HDL particles [61], with no free apo M detected in plasma [53]. HDL_M inhibits LDL oxidation and promotes cholesterol efflux [62, 63]. HDL_M carries endothelium-protective sphingosine-1-phosphate (S1P); [64] plasma apo M correlates with plasma total cholesterol [65]. Importantly, HDL-bound S1P levels predict the severity of coronary artery disease (CAD) [66], and HDL-S1P has salutary effects that are independent of lipoprotein metabolism, including enhanced cardioprotective nitric oxide-dependent vasodilation [64, 67, 68]. Notably, HDL from CVD patients vs. healthy controls is S1P-deficient [69]. Moreover, HDL-S1P-dependent signaling is impaired, and S1P content is reduced in CAD-HDL as compared to healthy HDL, an effect that is reversed by exogenous S1P even in apo M-deficient HDL [66].

  Studies of apo M in mice complement those in humans. Apo M transgenic mice have increased HDL-S1P. Given that S1P signaling maintains endothelial integrity [64, 70] and immune homeostasis [71], HDL-apo M may atheroprotect by transporting S1P to endothelial and immune cell receptors [72]. Apo M overexpression also increases HDL-C and atheroprotects, perhaps by enhanced preβ-HDL formation [73, 74] and MΦ cholesterol efflux [75]. S1P-deficiency is associated with endothelial gaps and vascular leak, which are central to inflammation, effects that are reversed when S1P levels are restored [76]. Apo M-null mice have low HDL-C and HDL-S1P. S1P

preferentially associates with HDL_M; thus, apo M likely irreversibly binds specific HDL particles thereby giving those particles high S1P affinity. The role of apo M in S1P delivery to the endothelium remains controversial for two reasons. First, incorporation of S1P into apo M-deficient HDL restores defective S1P signaling in vitro and in vivo [66]. Second, high affinity S1P binding by apo M would be expected to reduce S1P bioavailability and with it, its therapeutic value.

- Plasma PON1 (~44 kDa) catalyzes the hydrolysis of the organophosphate insecticide, paraoxon, an acetylcholine esterase inhibitor. PON1 is synthesized in the liver [77] and after secretion associates with HDL. PON1, which is also secreted by HepG2 cells [78], is associated with atheroprotection, attributed mainly to its inhibition of lipoprotein oxidation via hydrolysis of lipid hydroperoxides (Reviewed) [79, 80].
- Hpr (~45 kDa) and apo L1 (~42 kDa) are TLF components that give humans resistance to Tbb [81•]. Apo L1 occurs as a 42-kDa protein and a 39-kDa-truncated form, including its SP; both occur in HDL. Like apo M, free apo L1 is not detectable in plasma suggesting a similar high lipophilicity [82]. Whereas apo L1 is a trypanosome lytic factor, Hpr potentiates killing by increasing membrane permeability via a surface receptor in the parasite [83]. The retained SP mediates HDL assembly and function of Hpr [57••]. Hpr and apo L1 are secreted with SPs [84], which are cleaved in HEK cells [85] suggesting that these cells but not hepatocytes contain the appropriate peptidase. For unknown reasons, apo L1 gene variation underlies end-stage renal disease in blacks [86].

  Haptoglobin (HP), which occurs in plasma without its SP, has 90 % sequence identity with Hpr but does not occur on HDL, suggesting that the SP is the essential high affinity HDL-associating moiety in Hpr [85]. Occurrence of Hpr and apo L1 on a common particle is consistent with two hypotheses: (1) Both are nontransferable because they have highly lipophilic SPs; (2) Hpr and apo L1 have a shared if not common biogenic itinerary. It has also been suggested that the differential penetration of signal sequences of various proteins into PL bilayers determines the differential distribution of apo M, apo L1, Hpr, and PON1, into different HDL subclasses [57••].

Conclusions

Epidemiological data shows a strong negative correlation of plasma HDL-C levels with CVD risk. Historically, this was attributed to the role of HDL as an acceptor of macrophage cholesterol for transport to and disposal by the liver, i.e., reverse cholesterol transport. However, as new data have revealed the complexity of HDL composition, structure and function, and the heterogenous nature of HDL subspecies, it is apparent that the beneficial effects/functions of HDL are much more complex and have salutary effects that are independent of cholesterol transport. Numerous gaps remain in our understanding of the speciated HDL biogenesis and function, prompting the need for more studies to fully delineate the role of individual HDL species in HDL function. The complexity of HDL species will make the design of new therapeutics that enhances the cardioprotective effects of HDL, more challenging. Nevertheless, current studies of HDL biogenesis and RCT mechanisms should smooth the course.

Compliance with Ethical Standards

Conflict of Interest C. Rosales, W. S Davidson, and H. J. Pownall declare that they have no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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