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HDL in the 21st Century

A Multifunctional Roadmap for Future HDL Research

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ABSTRACT: Low high-density lipoprotein cholesterol (HDL-C) characterizes an atherogenic dyslipidemia that reflects adverse lifestyle choices, impaired metabolism, and increased cardiovascular risk. Low HDL-C is also associated with increased risk of inflammatory disorders, malignancy, diabetes, and other diseases. This epidemiologic evidence has not translated to raising HDL-C as a viable therapeutic target, partly because HDL-C does not reflect high-density lipoprotein (HDL) function. Mendelian randomization analyses that have found no evidence of a causal relationship between HDL-C levels and cardiovascular risk have decreased interest in increasing HDL-C levels as a therapeutic target. HDLs comprise distinct subpopulations of particles of varying size, charge, and composition that have several dynamic and context-dependent functions, especially with respect to acute and chronic inflammatory states. These functions include reverse cholesterol transport, inhibition of inflammation and oxidation, and antidiabetic properties. HDLs can be anti-inflammatory (which may protect against atherosclerosis and diabetes) and proinflammatory (which may help clear pathogens in sepsis). The molecular regulation of HDLs is complex, as evidenced by their association with multiple proteins, as well as bioactive lipids and noncoding RNAs. Clinical investigations of HDL biomarkers (HDL-C, HDL particle number, and apolipoprotein A through I) have revealed nonlinear relationships with cardiovascular outcomes, differential relationships by sex and ethnicity, and differential patterns with coronary versus noncoronary events. Novel HDL markers may also have relevance for heart failure, cancer, and diabetes. HDL function markers (namely, cholesterol efflux capacity) are associated with coronary disease, but they remain research tools. Therapeutics that manipulate aspects of HDL metabolism remain the holy grail. None has proven to be successful, but most have targeted HDL-C, not metrics of HDL function. Future therapeutic strategies should focus on optimizing HDL function in the right patients at the optimal time in their disease course. We provide a framework to help the research and clinical communities, as well as funding agencies and stakeholders, obtain insights into current thinking on these topics, and what we predict will be an exciting future for research and development on HDLs.

Key Words: atherosclerosis ■ biomarkers ■ inflammation ■ lipoproteins ■ lipoproteins, HDL

Epidemiologic evidence of an inverse association of plasma high-density lipoprotein cholesterol (HDL-C) levels with atherosclerotic cardiovascular disease (ASCVD) risk has not translated into positive outcomes in large-scale randomized controlled trials of interventions that raise HDL-C levels.[1] Lack of a plausible causal link between HDL-C and ASCVD from Mendelian randomization studies has led to a paradigm shift toward determining whether improving high-density lipoprotein (HDL) function explains the cardioprotective effects of HDLs. Another recent major shift in the HDL area has been toward identifying broader roles beyond atheroprotection, including infections and autoimmune disorders, diabetes, chronic kidney disease, and cancer, all of which are driven by inflammation and perturbed metabolism.

We aim to summarize the current understanding of the structure, function, and metabolism of HDLs, as well as their clinical usefulness as a potential therapeutic. The

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main recurring themes in the article include the diversity of HDL structure and function; the dynamic and context-dependent nature of HDL functions, especially with respect to acute and chronic inflammatory states; and the complexities of the molecular regulation of HDLs. These themes collectively highlight an increasing need for sophistication and validation in future studies. We hope that new and evolving insights into the beneficial functions of HDLs will pave the way for their use in both cardiovascular and noncardiovascular diseases. We provide a framework for the research and clinical communities, as well as for funding agencies and stakeholders, to become more informed and obtain insights into what we predict will be an exciting future for research and development on HDLs.

HDL STRUCTURE AND FUNCTION

HDL structure and function are inextricably linked; thus, it is essential to consider them both when investigating their roles in disease states.

HDL Structure

The HDLs in human plasma are predominantly spherical particles. They consist of several distinct HDL subpopulations of particles of varying size, surface charge, and lipid and apolipoprotein composition. This heterogeneity is a reflection of the remodeling of individual HDL subpopulations by plasma factors such as the cholesterol-esterifying enzyme lecithin:cholesterol acyl transferase (LCAT), CETP (cholesteryl ester transfer protein), phospholipid transfer protein, hepatic lipase, and endothelial lipase. Despite this heterogeneity, all HDLs have the same overall structure: a water-insoluble, neutral lipid core (mainly cholesteryl esters and some triglycerides) surrounded by a surface monolayer (mainly phospholipids and some unesterified cholesterol) in which apolipoproteins are embedded. HDL apolipoproteins are highly α-helical. These helices have a hydrophobic face that drives association with lipid as well as a hydrophilic face that confers water solubility on the HDL particles.

Detailed insights into the structural organization of spherical HDLs have emerged from studying homogeneous populations of reconstituted HDLs (rHDLs) and HDL subpopulations isolated from human plasma. Mass spectrometric analysis of these preparations indicates that most HDLs contain 3 copies of apolipoprotein A-I (apoA-I) that are organized on the particle surface as a trefoil[2] or as 2 copies in an antiparallel orientation with the third apoA-I molecule localized separately in a U-shaped conformation.[3] Whether these variations in the spatial organization of apoA-I on the HDL surface affect HDL function is unknown.

As discussed in detail in a later section, HDLs contain several other apolipoproteins in addition to apoA-I. The second most abundant HDL apolipoprotein is apoA-II, followed by apoA-IV, the C apolipoproteins, apoE, and apoM. These apolipoproteins all contribute to HDL structural stability and, in some cases, HDL function. HDLs also transport a cargo of other proteins that potentially further affect HDL function.

In addition to the aforementioned proteins that are predominantly associated with lipid metabolism, HDLs contain proteins that promote proteolysis (eg, α-1-antitrypsin), hemostasis (α-2-HS-glycoprotein), immunity (eg, the acute phase reactant SAA4), complement activation (eg, complement C3), and inflammation (eg, haptoglobin-related protein; https://homepages.uc.edu/~davidswm/HDLproteome.html). Because the concentration of most HDL-associated proteins is lower than the plasma concentration of HDL particles, each protein can associate only with specific HDL particle subsets.[4] We do not know whether the association of these proteins with HDLs is regulated by the size or composition of the particles or how HDL-associated proteins affect HDL function. The development of innovative approaches to address these issues will provide important insights into the interrelationship of HDL structure, function, and metabolism, and potentially identify targets that may boost the cardioprotective and other functions of HDL. Progress is being made in this direction with the clustering of specific HDL subpopulations into functional classes on the basis of their proteome.[4]

HDL Function

HDLs protect the function and survival of organisms by multiple, overlapping mechanisms. For example, proteins and bioactive lipids that associate with HDLs directly activate signal transduction pathways. HDLs also function indirectly by effluxing cholesterol from cells and influencing cholesterol homeostasis. HDLs can detoxify potential hazards through enzymes such as paraoxonases or by delivering them to the liver for biotransformation and excretion by pathways that are shared with reverse cholesterol transport.

Cholesterol Efflux and Reverse Cholesterol Transport

The efflux of excess cholesterol from macrophages and other cell types is the most extensively studied function of HDLs and apoA-I. Large, spherical HDLs accept the cholesterol that is exported from cells by the adenosine triphosphate–binding cassette transporter G1 (ABCG1); while the related transporter, adenosine triphosphate–binding cassette transporter A1 (ABCA1), exports cellular cholesterol to lipid-free apoA-I and small, dense HDLs. The importance of cholesterol efflux has emerged from human cohort studies that have revealed inverse associations between the capacity of plasma depleted of apoB-containing lipoproteins to accept macrophage-derived cholesterol and cardiovascular risk in most, but not all, studies.[5]

The efflux of cholesterol to HDLs and apoA-I also represents the first step in reverse cholesterol transport, the pathway whereby excess cholesterol from macrophages in the artery wall is acquired by apoA-I and HDLs and transported to the liver for excretion as a component of bile. Preclinical reverse cholesterol transport studies have established that increasing cholesterol flux though the reverse cholesterol transport pathway reduces atherosclerosis in animal models. Although clinical translation of these findings has been slow, a study using an integrated approach to quantify the entire reverse cholesterol transport pathway in humans was recently published.[6] This may pave the way for detailed investigations of the regulation of reverse cholesterol transport and ASCVD by HDLs in humans.

Inhibition of Inflammation by HDLs

HDLs reduce inflammation in multiple cell types, including endothelial cells and macrophages. In endothelial cells, HDLs inhibit inflammation by reducing activation of nuclear factor κB (NF-κB) and 3β-hydroxysteroid-Δ24 reductase, by activating the cytoprotective enzyme heme oxygenase-1 and by inhibiting inflammasome activation.[7,8] HDLs exert these effects by several mechanisms, including the interaction of HDL-associated apoM/sphingosine-1-phosphate (S1P) with S1P receptors.[9] They also reduce inflammation in monocytes and attenuate the binding of monocytes to adhesion molecules on the surface of activated endothelial cells.[9,10] Collectively, these findings highlight several targets with the potential to improve the anti-inflammatory properties of HDLs in endothelial cells. The role of HDLs in macrophage inflammation is addressed in the next section.

Antidiabetic Properties of HDLs

HDLs and HDL apolipoproteins improve glycemic control in animal models of diabetes by enhancing pancreatic β-cell function and survival and improving insulin sensitivity.[11] HDLs also inhibit β-cell apoptosis and protect β cells from oxidation by low-density lipoproteins (LDLs).[12,13]

Evidence that the antidiabetic functions of HDLs are clinically relevant has emerged from a post hoc meta-analysis of all CETP inhibitor trials showing a 12% reduction in incident diabetes.[14] A more detailed analysis of dalcetrapib revealed that the reduced incidence in diabetes may be a consequence of the treatment-related increase in HDL-C (but not change in body mass index) and regression from diabetes to no diabetes.[15] These studies provide the first direct evidence that increasing HDL levels may reduce cardiometabolic risk. Identification of specific HDL subpopulations that mediate these effects would enable this approach to be further developed through commercial production of relevant rHDLs.

Inhibition of Oxidative Stress by HDL

HDLs reduce oxidative stress in LDLs and other atherogenic lipoproteins by accepting lipid hydroperoxides and detoxifying them into lipid hydroxides that are cleared from the circulation by the liver. Small HDLs inhibit oxidation more effectively than large HDLs.[16] This can potentially negate, at least in part, the reduced cardioprotection that is associated with low plasma HDL levels. It also raises the possibility that treatment with HDL-raising agents that increase the level of large HDLs may not improve the antioxidant properties of HDLs.

Paraoxonase 1 and platelet-activating factor acetyl hydrolase contribute to the antioxidant properties of HDLs independent of reducing lipid hydroperoxides to hydroxides. Although paraoxonase 1 knockout mice are atherosclerosis-prone, and overexpression of human paraoxonase 1 reduces atherosclerosis in mice,[17,18] the mechanism by which paraoxonase 1 inhibits oxidation, and its effect on atherosclerotic lesion development, is not well understood. The mechanism of the antioxidant properties of platelet-activating factor acetyl hydrolase have, by contrast, been elucidated, and include the hydrolysis of oxidized fatty acid constituents in phospholipids. However, the precise contribution of platelet-activating factor acetyl hydrolase to the antioxidant properties of HDLs requires further clarification. Interventions that exploit these cardioprotective functions of HDLs have yet to be developed.

Conclusions

Insights into the protective functions of HDLs have progressed in recent years, but little is known about the identity and structural characteristics of specific HDL subpopulations that mediate these effects. The development of innovative approaches to identify these subpopulations, their origins, and their regulation would enable specific HDL subpopulations to be targeted as a means of preserving, and possibly enhancing, the cardioprotective functions of HDLs. Recent technological advances have increased the feasibility of reaching this milestone in the short to medium term and could ultimately have major long-term benefits for the clinical usefulness of HDL-based therapies.

HDL AND INFLAMMATION

Inflammation is a key driver of chronic diseases such as ASCVD and diabetes as well as infections and malignancies. HDLs directly affect the inflammatory process, and inflammation affects HDL function.[8,19–25] Approximately 90% to 95% of the apoA-I in plasma is bound to HDL particles, but proinflammatory states can cause it to dissociate into the circulation in a lipid-free or lipid-poor form.[26] The role of HDLs and apoA-I in macrophage inflammation, a key driver of atherosclerotic lesion progression, has been investigated extensively.[8,10,19–25,27] Early studies have focused on the anti-inflammatory effects of HDLs,[8,10,19,20,23–25,27] but more recent studies showing that HDLs and apoA-I can also be proinflammatory[20–22] may

be 1 explanation for the limited success of HDL-raising drugs in reducing ASCVD. However, the proinflammatory effects of HDLs could be beneficial in other diseases, such as sepsis, where enhanced inflammation may promote efficient clearance of bacteria.[22] Understanding the mechanistic links between HDLs and their role in inflammation is vital for understanding the prognostic and therapeutic potential of this lipoprotein class.

Whether HDLs and apoA-I are proinflammatory or anti-inflammatory depends on several structural/functional features, including HDL composition,[20,21] macrophage cholesterol content,[20] and signaling pathways.[19,22] Macrophages produce pro- and anti-inflammatory cytokines. Production of proinflammatory cytokines occurs downstream of Toll-like receptors (TLRs) that are activated by components of viruses or bacteria. The best known examples of this are lipopolysaccharide, which activates TLR4, and lipoteichoic acid, which activates TLR2.[28] Whereas HDLs and apoA-I suppress inflammation by binding directly to and neutralizing lipopolysaccharide or lipoteichoic acid,[28] they also affect TLR activation and downstream signaling pathways directly (Figure 1).

Cholesterol Efflux-Dependent Anti-Inflammatory Effects of HDLs

HDLs suppress inflammation in macrophages by decreasing TLR4 signaling that is mediated by MyD88 (myeloid differentiation primary response 88)/NF-κB or TRIF (TIR-domain–containing adapter-inducing interferon-β), which reflect early and late anti-inflammatory responses, respectively (Figure 1A1 and 1A2).[20,24,25] These effects are related partly to the ability of HDLs to accept the cholesterol that effluxes from cells through ABCA1 and ABCG1 in processes that suppress TLR4 expression on cell surfaces.[20,24] The HDL-mediated suppression of proinflammatory responses in macrophages that occurs when lipopolysaccharide binds to TLR4 is dependent partially on expression of the *Abca1* and *Abcg1* genes (Figure 1A1).[29] The ability of apoA-I, but not HDLs, to suppress proinflammatory monocyte activation by PMA (phorbol 12-myristate 13-acetate) in human monocytes also depends on ABCA1.[10] Humans heterozygous for loss-of-function mutations in the *ABCA1* gene have a ≈50% decrease in plasma HDL-C levels, increased plasma proinflammatory cytokine levels, and extensive vascular inflammation compared with healthy controls,[30] suggesting clinical relevance.

Cholesterol Efflux-Independent Anti-Inflammatory Effects of HDLs

The anti-inflammatory effects of HDLs are related to suppression of lipopolysaccharide-induced gene expression downstream of the TRIF and TRIF-related adaptor molecule.[25] Mechanistically, HDLs reduce the availability of TRIF for signaling by translocating the TRIF-related adaptor molecule from the plasma membrane to intracellular compartments (Figure 1A2).[25] HDLs also inhibit inflammation by increasing expression of activating transcription factor 3 (Atf3), which is induced by TLRs, but limits proinflammatory cytokine production downstream of NF-κB.[19] None of these effects is related to cholesterol efflux (Figure 1A3).[19] However, the Atf3 findings are somewhat controversial. Some studies have reported that HDLs do not increase *Atf3* gene expression[8,20,22]; others have found that HDLs suppress *Atf3* gene expression through TLR4 in a process that depends on cholesterol efflux (Figure 1A1).[20] In contrast, cholesterol efflux does not affect TLR9 signaling.[24] This may explain why ATF3 accounts for the anti-inflammatory effects of HDL when TLR9 is activated (Figure 1A3).

Proinflammatory Effects of HDLs

The apoA-I that dissociates from HDLs under proinflammatory conditions[26] directly activates TLR2 and TLR4 (Figure 1B4).[21] Although controversial,[20] HDLs may also exert proinflammatory effects by augmenting protein kinase C activation in response to TLR ligands (Figure 1B5).[22] To a large extent, the proinflammatory effects of HDLs are attributable to excessive cellular cholesterol depletion. This activates IRE1α (inositol-requiring enzyme 1α)/ASK1 (apoptosis signal-regulating kinase 1)/p38 MAPK (p38 mitogen-activated protein kinase) signaling, which results in a proinflammatory endoplasmic reticulum stress response (Figure 1B6).[20]

HDLs and Cholesterol Efflux Pathways Suppress Inflammasome Activation

Inflammasomes are intracellular complexes comprising the sensor molecule NOD-like receptor, the adaptor protein ASC (apoptosis-associated speck-like protein), and caspase-1.[31] NLRP3 (NOD-like receptor family pyrin domain containing 3), the most extensively characterized inflammasome, requires 2 signals for activation: NF-κB activation, which increases transcription of all NLRP3 inflammasome components, called inflammasome priming; and other signals[31] including, but not limited to, accumulation of free cholesterol or cholesterol crystals in lysosomes, which leads to cleavage of caspase-1[32] and generates the proinflammatory cytokines interleukin-1β and interleukin-18.[31]

HDLs suppress inflammasome activation by decreasing lipopolysaccharide-induced inflammasome priming.[8] ABCA1- and ABCG1-mediated cholesterol efflux also suppresses inflammasome activation in myeloid cells and dendritic cells, which reduces atherosclerosis and autoimmunity, respectively.[27,33] Patients with Tangier disease

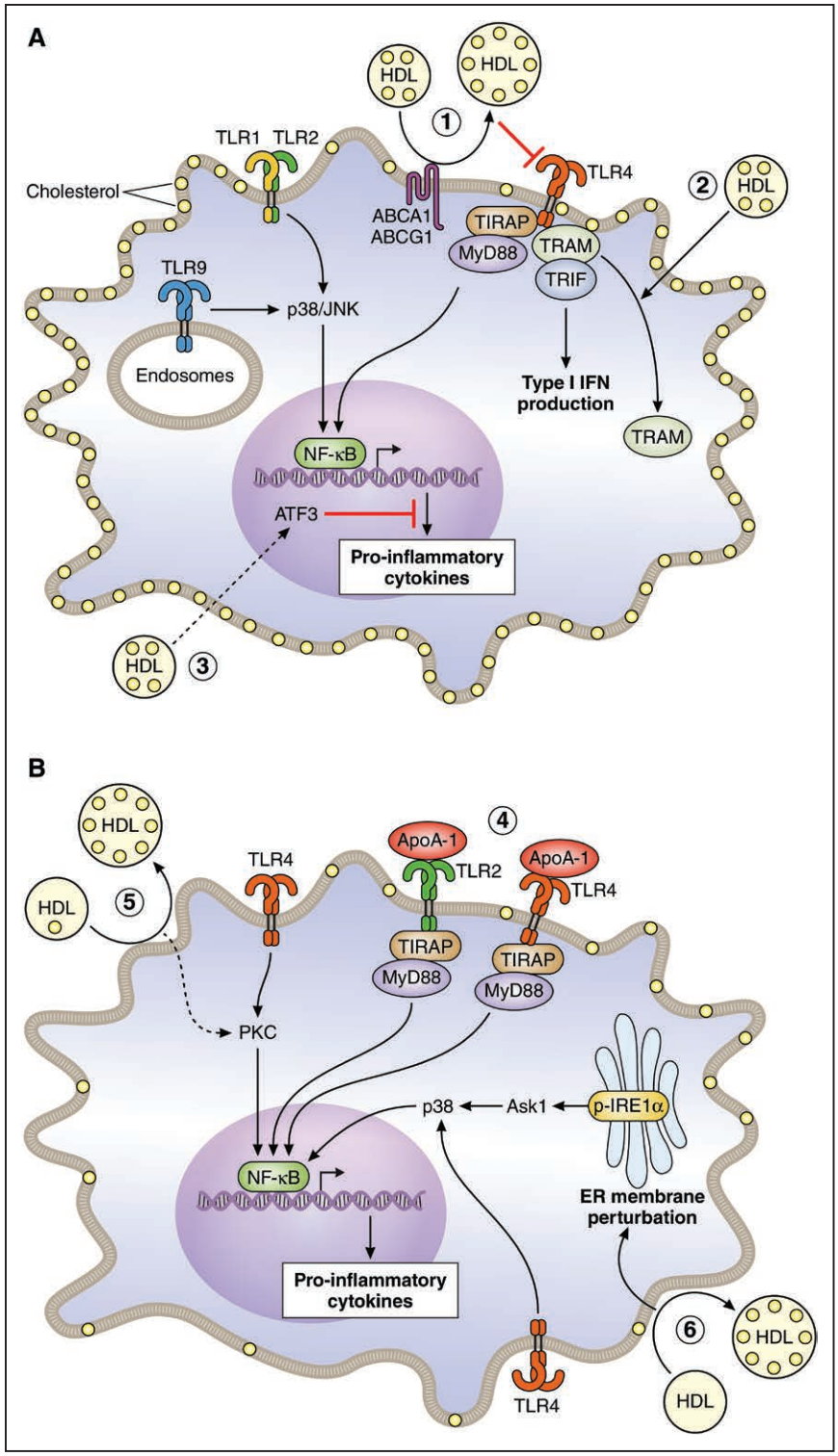

Figure 1. Anti- and proinflammatory effects of HDL in macrophages. **A**, Anti-inflammatory effects. 1: HDL induces cholesterol efflux mediated by the cholesterol transporters adenosine triphosphate (ATP)–binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1), leading to decreased Toll-like receptor (TLR) 4 surface expression and decreased downstream MyD88 (myeloid differentiation primary response 88) and TRIF (TIR-domain–containing adapter-inducing interferon-β) signaling, suppressing nuclear factor κB (NF-κB) and type I interferon (IFN) response, respectively. 2: HDL stimulates the translocation of TRIF-related adaptor molecule (TRAM) from the plasma membrane to intracellular compartments, reducing its availability for TRIF signaling and diminishing type I IFN production. 3: HDL induces activating transcription factor 3 (Atf3) expression, suppressing TLR9 or TLR1/2-induced inflammatory gene expression downstream of NF-κB. The dashed arrow indicates that the exact mechanism is unknown. Yellow dots indicate free cholesterol and in the case of HDL, particle free cholesterol enrichment because of cholesterol efflux. **B**, Proinflammatory effects. 4: Apolipoprotein A (ApoA)–1 binds to TLR2 and TLR4, enhancing MyD88 signaling and NF-κB activation and TRIF signaling (not shown). 5: HDL induces plasma membrane cholesterol depletion, augmenting protein kinase C (PKC) signaling and downstream NF-κB activation induced by a TLR ligand. 6: HDL induces excessive cholesterol depletion, leading to endoplasmic reticulum (ER) membrane perturbation and enhanced IRE1α (inositol-requiring enzyme 1α)/Ask1 (apoptosis signal-regulating kinase 1)/p38 MAPK (p38 mitogen-activated protein kinase) signaling, which augments NF-κB activation in the presence of lipopolysaccharide (shown as TLR4 activation). HDL indicates high-density lipoprotein; and NK, c-Jun N-terminal kinase.

who are homozygous for loss-of-function mutations in the *ABCA1* gene have very low cholesterol efflux and plasma HDL-C levels and increased plasma levels of interleukin-1β and interleukin-18, indicating human relevance.[27] Similarly, decreased cholesterol efflux to HDLs attributable to reduced gene expression of *ABCA1/ABCG1* in blood monocytes of patients with poorly controlled diabetes,[34] chronic kidney disease,[35] or rheumatoid arthritis[36] may contribute to inflammasome activation and increased systemic inflammation.

Conclusions

HDLs and apoA-I have pro- and anti-inflammatory effects. The anti-inflammatory effects are beneficial in the context of atherosclerosis and diabetes; the proinflammatory

properties of HDLs and apoA-I may contribute to efficient clearance of bacteria in sepsis. Some of the effects of HDLs and apoA-I on inflammation are dependent on cholesterol efflux. Cholesterol efflux to HDLs and apoA-I is generally anti-inflammatory in monocytes and macrophages. However, excessive cholesterol depletion in macrophages by HDLs can induce proinflammatory effects.

APPLICATION OF -OMICS TO HDLS

Genomics

Mendelian randomization studies focusing primarily on the cholesterol content of HDLs have not found a causal link between HDL-C and ASCVD[1] but have suggested a causal role for HDLs in chronic kidney disease and infection.[1]

Mendelian randomization studies are hampered by several major limitations. First, they rely on linear associations between exposure and outcome. However, the associations of HDL-C with the risk of ASCVD, chronic kidney disease, infection, and mortality are parabolic.[1] Within the broad nadirs encompassing up to 40% of the population, differences in HDL-C are not associated with any change in risk.[1] Second, the directions of associations are not consistent. Loss-of-function mutations in *SCARB1* (the gene that encodes for SR-BI [scavenger receptor class B type I]) and *APOA1* increase and decrease HDL-C, respectively, but both increase the risk of ASCVD.[37,38] Third, the cholesterol in HDL is an inert surrogate marker of the number and size of HDL particles and is not responsible for any of the cardioprotective functions of HDLs. Thus, genomic approaches investigating markers of HDL function are likely to yield more biologically and clinically relevant insights than HDL-C levels. Targeting apoA-I may also directly link genes to function. However, a recent Mendelian randomization study did not find a genetic association of apoA-I plasma levels with risk of ASCVD.[39] Mendelian randomization studies have linked plasma levels of apoC-III and risk of ASCVD, but this is typically interpreted as an adverse role of apoC-III in triglyceride-rich lipoprotein metabolism rather than an indicator of HDL dysfunction.[40] Additional Mendelian randomization studies on apoC-III levels in HDL or apoB-free plasma are needed to demonstrate whether the association of apoC-III–containing HDL with increased risk of ASCVD is causal.[41]

Proteomics

Reports on the number of HDL-associated proteins vary from 9 to nearly 500.[42] This reflects differences in how the HDLs were isolated, the sensitivity of the mass spectrometry method that was used to detect individual proteins, and the absence or presence of disease.[42]

The method of HDL isolation will also affect the number of HDL-associated proteins. Isolating HDLs by sequential ultracentrifugation suffers from contamination with LDLs, exosomes, and microvesicles. High-resolution size exclusion chromatography combined with phospholipid affinity[42] or anti-apoA-I immunoaffinity chromatography[4,43] go some way toward reducing this risk. To date, 219 HDL-associated proteins have been validated by at least 3 independent laboratories (https://homepages.uc.edu/~davidswm/HDLproteome.html).[44] The wide range of protein concentrations (<100 nmol/L for apoL1 and PLTP to >50 μmol/L for apoA-I relative to an average HDL particle concentration of ≈20 μmol/L; Figure 2) suggests that HDL-associated proteins are nonrandomly distributed among HDL particles. A recent approach using 2 sequential immunoaffinity chromatography steps supports this concept, revealing 16 HDL subclasses with distinct proteomes.[4]

Posttranslational modifications of HDL-associated proteins such as acylation and phosphorylation, and modifications such as oxidation, nitration, chlorination, and carbamylation that are caused by inflammation, hyperglycemia, and uremia, also have a profound effect on the HDL proteome.[42,44] A recent analysis by high-resolution mass spectrometry identified nearly 1000 different posttranslational modifications of 54 HDL-associated proteins, with apoA-I most frequently affected. Some of these modifications interfere with HDL function, including cholesterol efflux, and may serve as biomarkers of dysfunctional HDL.[42,44]

In line with these observations, future investigations of the effects of HDL-associated proteins on HDL function will need to account for alterations of the HDL proteome in response to disease or treatment[42] (Table 1).

Understanding of these complex changes in the HDL proteome with HDL function requires comprehensive high-throughput proteomic HDL assays. This has led to the development of a proteomic score derived from apolipoproteins A-I, C-I, C-II, C-III, and C-IV that correlates with cholesterol efflux capacity and independently associates with ASCVD and cardiovascular mortality.[70] Another recent proteomic study found that associations with ASCVD events were modified by either enrichment or depletion of HDLs with several proteins. ApoC-III enrichment of HDLs interferes with their capacity to inhibit apoptosis of endothelial cells, reduces their capacity to efflux cholesterol from macrophages, and is a potent adverse ASCVD risk marker.[41,49,70] This identifies apoC-III as an interesting HDL-targeted strategy for therapy beyond lowering of triglycerides.

Lipidomics

The concentrations of lipid species in plasma and HDLs range from nanomolar to millimolar, with cholesterol

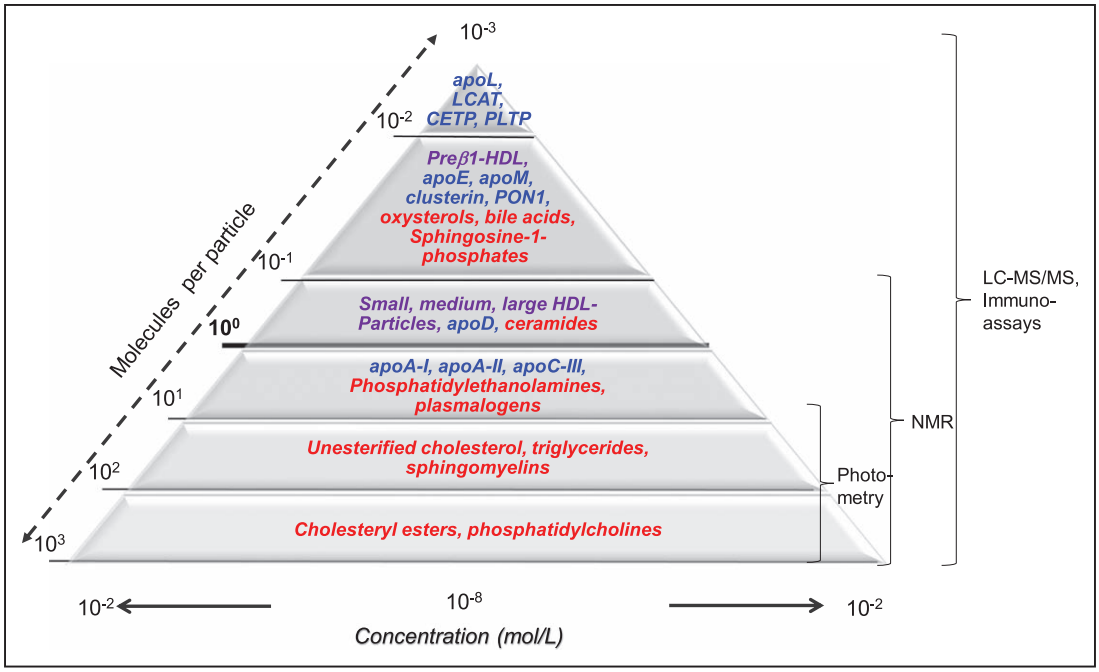

Figure 2. Concentration ranges of HDL particles as well as the different proteins and lipids found in HDL.
The width of the triangle's baseline axis reflects the concentrations of HDL particles and components, which range from >1 mmol/L (ie, 10^{-3} mol/L for cholesterol) to the submicromolar range (ie, 10^{-7} or 10^{-8} mol/L) for lipids such as sphingosine-1-phosphates and oxysterols or proteins such as apoL1 (apolipoprotein L1) or PLTP (phospholipid transfer protein). The numbers on the left diagonal axis of the triangle show the abundance of HDL subclasses or components relative to an average concentration of HDL particles of 20 µmol/L, which is highlighted with the bold line crossing the triangle at $10^0 = 1$. The brackets on the right side of the triangle reflect the measuring ranges of analytical methods used for the characterization of HDL. MicroRNAs are not presented but their concentration of 10 000 copies/µg HDL protein[82] implies a relative abundance of about 1 molecule per 10^7 HDL particles. Apo indicates apolipoprotein; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LCAT, lecithin:cholesterol acyltransferase; NMR, nuclear magnetic resonance; and PON1, paraoxonase 1.

being the most abundant (>100 molecules/HDL particle) and other lipids such as S1P, oxysterols, and bile acids at extremely low abundance (1 molecule associated with <10% of HDL particles; Table 1 and Figure 2). Cholesteryl esters and triglycerides in the core influence HDL function indirectly by regulating particle size and being substrates for esterases. Lipids in the HDL surface influence HDL function directly: the fatty acid acyl chains in phospholipids and sphingomyelin influence particle fluidity and rigidity, respectively, and thereby HDL function, including cholesterol efflux[45]; S1P has vasoprotective and antidiabetic functions[63,64]; and some HDL lipids are substrates for enzymes that generate biologically active lipids including lysophospholipids and polyunsaturated fatty acids.[71] These functions are interconnected. For example, HDLs promote the efflux of cholesterol and bioactive lipids such as S1P and oxysterols.[12,72] By providing substrates and inducing efflux, HDLs thus serve as a scaffold for paracrine regulation by bioactive lipids. Standard analysis of isolated HDLs may therefore underestimate the complexity and functional relevance of the HDL lipidome in vivo.

Variations in the structure and concentration of HDL lipids precludes, to some extent, the development of a comprehensive, unifying standardized method for measuring all lipid species. Nuclear magnetic resonance (NMR) spectrometry directly measures individual lipoprotein subclasses without previous fractionation and lipid extraction. However, its low sensitivity allows measurement only of lipid subclasses (cholesteryl esters, unesterified cholesterol, phosphatidylcholines, sphingomyelins) rather than individual lipid species. Nevertheless, NMR-derived HDL particle numbers predict incident ASCVD events better than HDL-C.[46] Broad NMR profiling of HDLs also correlates with cholesterol efflux capacity, but not other HDL functions.[45] NMR studies found that type 2 diabetes is positively associated with small HDLs and inversely associated with large HDLs,[47] whereas ASCVD is inversely associated with small and medium-sized HDLs.[45] In contrast with NMR, mass spectrometry has higher sensitivity at low concentrations and detects more lipid species with potential functional as well as clinical relevance; for example, lysophosphatidylcholines and sphingomyelins differing by the composition of O- and N-linked fatty acids (Table 1).[45,61,73] Several of them have been associated with the presence of acute or chronic ASCVD or diabetes and react to therapeutic interventions.[58–60] Prospective studies are needed to investigate the prognostic performance of specific lipid species in HDL in addition to total plasma or serum.

Table 1. Examples of HDL-Associated Proteins, Lipids, and miRs Associated With the Presence or Incidence of Diseases or Mortality

Molecules	Function in HDL	Disease association
Particles		
HDL particle number	Determines CEC[45]	Inversely with risk of ASCVD[46]
Small HDL	Determines CEC[45]	Positively with diabetes, inversely with risk of ASCVD[45,47]
Large HDL	Determines CEC[45]	Positively with diabetes[45,47]
Pre-β HDL	HDL precursor, determines CEC	Positively with the presence of ASCVD[48]
Proteins		
ApoA-I	Structural component	Total apoA-I: inversely with incident ASCVD or mortality[39]; various posttranslational modifications of apoA-I are positively associated with presence of ASCVD[42,44]
	Activator of LCAT	
	Ligand of HDL receptor and ABCA1	
	Antioxidative	
ApoC-III	Promotes apoptosis of endothelial cells and activation, inhibits cholesterol efflux[49,50]	Positively with incident ASCVD or diabetes[39,51]
ApoE	Hepatic removal of HDL,[52] stimulation of cholesterol efflux anti-inflammatory activities	Inversely with risk of ASCVD and dementia[1]
Serum amyloid A	Acute phase reactant inhibits cholesterol efflux and eNOS activation[53]	Positively with presence of ASCVD and mortality of patients with ASCVD or ESRD[53,54]
Paraoxonase 1	Inhibition of lipid peroxidation	Inversely with presence of ASCVD or diabetes[55]
Pulmonary surfactant protein B	Component of lung surfactant	Positively with mortality in patients with ESRD or heart failure[54,56]
Lipids		
Cholesteryl esters	Core lipid determining size and shape	Inversely with presence and incidence of ASCVD, diabetes, and other diseases[1]
Triglycerides	Core lipid	Positively with mortality in ASCVD[57]
Phosphatidylcholines	Determine fluidity of HDL and thereby cholesterol efflux capacity[45,58]; substrate for the generation of lysophospholipids	Heterogeneous, depending on species[45,58]
PC and PE plasmalogens	Antioxidative[58–60]	Inversely with presence of ASCVD or diabetes[58–60]
Lysophosphatidylcholines (eg, LPC18:1, LPC18:2)	Enzymatically produced from HDL-derived lipids; signaling	Inversely with diabetes[45,61]
Sphingomyelins	Determine rigidity of HDL and thereby cholesterol efflux capacity and antiapoptotic activity toward endothelial cells[45]; substrate for the generation of S1P and ceramides	Some species inversely with presence of diabetes or ASCVD[45,61,62]
Sphingosine-1-phosphate	Agonist of 5 G-protein–coupled S1P receptors; multiple vasoprotective, antidiabetic, and anti-inflammatory actions[63,64]	Inversely with presence of ASCVD or diabetes[64]
microRNAs		
miR-223	Most abundant miRNA in HDL; regulates VCAM expression in endothelial cells and cholesterol metabolism in liver[65]	Increased in ACS and diabetes; decreases with weight loss[66,67]
miR-375-3p	Secreted by pancreatic β cells to HDL[68]	Increased levels in β-cell failure after islet transplantation[69]

ABCA1 indicates adenosine triphosphate–binding cassette transporter A1; ACS, acute coronary syndrome; Apo, apolipoprotein; ASCVD, atherosclerotic cardiovascular disease; CEC, cholesterol efflux capacity; eNOS, endothelial nitric oxide synthase; ESRD, end-stage renal disease; HDL, high-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; miR, microRNA; PC, phosphatidylcholine; PE, phosphatidylethanolamine; S1P, sphingosine-1-phosphate; and VCAM, vascular cell adhesion molecule.

Transcriptomics

In addition to proteins and lipids, RNAs are highly abundant in HDLs, the majority of which are fragments from longer RNAs originating from bacteria and fungi and being of unclear clinical relevance.[74] HDLs also transport microRNAs (miRs) that regulate cellular differentiation, proliferation, apoptosis, and metabolic homeostasis.[65,74] miR-223, the most abundant and best characterized miRNA in HDLs, is released from myeloid cells and delivered by HDL into endothelial cells, hepatocytes, smooth muscle cells, and monocytes.[75] To date, the functional consequence of HDL-derived miRs remains controversial.[76] The concentration of the most abundant HDL-associated miR-223 is 7 to 8 orders of magnitude lower than the plasma HDL concentration (20 μmol/L).[66,67,77] To deliver sufficient amounts of specific miRNAs for posttranscriptional regulation, HDLs may shuttle miRs secreted by circulating blood cells or neighboring cells and thereby mediate autocrine or paracrine rather than endocrine regulation. Only small case–control studies

have investigated the associations of HDL-associated miRNAs with disease and treatment.[66–69] Future studies should provide mechanistic insights and observations in large human cohorts.

Conclusions

Hypothesis-free research strategies have led to the discovery of genes, proteins, lipid species, and miRs that regulate HDL metabolism and determine the physiologic or pathologic functions of HDLs. Some of these HDL constituents may serve as biomarkers to improve the identification, treatment stratification, and monitoring of individuals at risk for different diseases. These multiple regulatory factors will necessitate the application of multiparametric -omics technologies to well-characterized biobanks in order to validate the usefulness of biomarker candidates. Specific and comprehensive analyses of HDL constituents by methods combining high resolution and high throughput, however, remain the main technical challenge in this field.

HDL: CLINICAL IMPLICATIONS

Clinical Implications in ASCVD

Low levels of HDL-C, specifically <40 mg/dL, are strongly associated with increased risk of coronary and peripheral arterial disease and are characterized by an atherogenic dyslipidemia consisting of high levels of small, dense LDL particles, elevated triglycerides, and increased insulin resistance. The remarkably consistent predictive information captured by low HDL-C, particularly among White populations, supports continued measurement of HDL-C clinically for diagnosing metabolic syndrome and as a guide for ASCVD risk prediction.

Despite the clinical usefulness of low HDL-C, the associations between HDL-C and ASCVD are not linear and vary according to race/ethnicity. In particular, there is a U-shaped association between HDL-C and ASCVD/mortality, with a linear inverse association preserved <40 mg/dL in men and <50 to 58 mg/dL in women, no association across the normal range (40 to 96 mg/dL in men and 50 to 134 mg/dL in women), and a modest but increased ASCVD risk at HDL-C levels >90 mg/dL in Asian populations, >97 mg/dL in White men, and >135 mg/dL in White women.[1,78] The links between HDL-C and ASCVD among the Black population may be attenuated or even trend in the opposite direction compared with the White population.[46] Future studies may clarify consistency of these associations across non-White populations and in noncoronary vascular domains.

ApoA-I is mechanistically linked to atherosclerosis and is inversely associated with ASCVD risk. However, apoA-I levels do not improve risk prediction beyond non-HDL-C and HDL-C levels,[79] limiting clinical usefulness as a prognostic biomarker. Other HDL apolipoproteins such as apoA-II and apoC-III may improve risk information,[4,41,80] but will require validation in longitudinal cohorts and usefulness beyond non–HDL-C and other standard risk factors. Given the mechanistic role for apoA-I in nonatherosclerotic cardiovascular disease (CVD), future studies should also assess whether apoA-I improves risk prediction of heart failure[81] and arrhythmias[82] and whether interventions targeting these diseases work, at least in part, by increasing apoA-I levels or function.

HDL-P reflects total HDL particle concentration and is measured commercially by NMR and ion mobility assays. HDL-P measured by NMR consistently outperforms HDL-C in associating with ASCVD in large cohorts.[46] For HDL-P to gain traction as a clinical risk marker, studies are needed that directly assess risk prediction for the specific vascular endpoints of myocardial infarction, stroke, and peripheral arterial disease. It is also important to establish whether HDL-P is useful for risk prediction in non-White populations.

HDL function is increasingly being used in observational and interventional investigations. Studies assessing ex vivo cholesterol efflux from macrophages to apoB-depleted serum have linked impaired efflux to a higher risk of incident and recurrent ASCVD in most, but not all, studies.[5] This risk may be specific to coronary atherosclerosis, especially thin-cap fibroatheroma and noncalcified plaque,[83–85] and has little to no relevance for cerebrovascular atherosclerosis and ischemic stroke.[86,87] A recent substudy of the PREDIMED trial (Prevención con Dieta Mediterránea) suggests that assessing multiple aspects of HDL composition and function gives insights into the effects of an intervention and whether the intervention is a potentially bone fide therapeutic target.[88] These assays are only research tools and need to be scaled up before they can be used clinically to improve risk prediction of incident cardiovascular events or for rapid, high-throughput measurement of HDL function in specific populations on the basis of risk status, sex, and ethnicity.

Given the dynamic nature of HDL structure–function relationships in the context of various diseases and therapies and the diversity of cardiovascular end points, several factors should be considered in the design and analysis of future epidemiologic, translational, and intervention studies focused on ASCVD phenotypes and outcomes (Table 2).

Clinical Implications in Noncardiovascular Diseases

HDL metabolism is directly relevant to noncardiovascular diseases such as cancer and diabetes. Epidemiologic studies have linked lower HDL-C and apoA-I levels to increased risk of lung, liver, colorectal, breast, prostate, and hematologic malignancies,[89] as well as

Table 2. Factors to Consider in Future Studies Investigating HDL Markers With Respect to Study Populations and Cardiovascular End Points

Factors
Type of CHD*
Stable CHD
Unstable angina
NSTEMI
STEMI
Coronary vs peripheral arterial disease
MI
Cerebrovascular atherosclerosis/ischemic (nonembolic) stroke
Lower extremity peripheral arterial disease
Atherosclerotic vs nonatherosclerotic CVD
Atherosclerotic CVD
CHD, cerebrovascular disease (atherosclerotic stroke, embolic stroke, hemorrhagic stroke), peripheral arterial disease
Nonatherosclerotic CVD
Heart failure, arrhythmias, vascular stiffness
Accounting for cardiometabolic medications
Lipid-modifying drugs
Glucose-lowering drugs
Blood pressure drugs (affecting vascular tone)

CHD indicates coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoprotein; MI, myocardial infarction; NSTEMI, non–ST-segment–elevation myocardial infarction; and STEMI, ST-segment–elevation myocardial infarction.

*Represents a continuum of risk but also reflects varying pathology from chronic atherosclerotic and inflammatory processes to increased thrombotic and acute inflammatory perturbations.

increased disease progression and diminished therapeutic response.[90] Higher HDL-C and apoA-I levels during treatment also predict improved response and survival.[91] These studies are balanced by some reports that specific cancers may upregulate apoA-I, particularly in metastatic disease,[92] perhaps reflecting increased cholesterol uptake by malignant cells during tumor progression. In line with these observations, SR-BI, which primarily mediates uptake of cholesteryl esters into cells, is upregulated in several cancers and prognostic for tumor progression and metastasis.[93] Although preclinical studies suggest a role for apoA-I in suppressing tumors, it remains unknown whether increasing apoA-I improves cancer prognosis in humans.

Specific questions to clarify the role of HDL markers include which HDL markers best predict risk of incident cancer versus progression versus prognosis. How does chemotherapy, especially immune checkpoint inhibitors, affect HDL function, and are these effects beneficial or harmful? To what degree do changes in HDL markers reflect the cancers themselves, the effects of treatments, changes in cancer-related cardiovascular risk, or changes in diet/lifestyle and body composition that are related to cancer treatment and survival?

The prevention of diabetes and its complications is a clear unmet need given its increasing prevalence. As described previously, a large body of preclinical data supports a protective role of HDLs in preventing hyperglycemia.[11] Therapies that increase HDL-C and apoA-I levels, such as CETP inhibitors and rHDL infusions, reduce the incidence and progression of diabetes.[14] Although these specific therapies have not translated to clinical use, it is clearly worthwhile to investigate whether interventions that target HDL metabolism may be used for the prevention and treatment of diabetes. The antioxidant and anti-inflammatory functions of HDLs are also decreased in patients with diabetes, who have a reduced ability to increase nitric oxide production, which may explain at least part of the increased cardiovascular risk in these patients.

Other noncardiovascular diseases in which there is emerging evidence of a role for HDLs include infection, renal failure, autoimmune disorders, age-related macular degeneration, and pulmonary hypertension.[1]

Future Directions

Future studies assessing HDL-C as a risk marker should consider the potential for nonlinearity as well as effects of ethnicity, prevalent disease status, and HDL functionality. The relationship of HDLs and apoA-I with nonatherosclerotic CVD diseases are poorly understood, reflecting a clear unmet need. Future studies on HDL function should focus on clinical effects of posttranslational and disease-specific modifications of apoA-I as well as the effect of other HDL-associated proteins and lipids. Total HDL particle concentration may give additional information on risk, but a lack of standardization limits the broader clinical usefulness of this approach. Use of composite end points (ie, myocardial infarction + stroke; all CVD) in epidemiologic and intervention studies may also blur associations, which highlights the importance of reporting end point–specific associations and effects. Use of apoA-I as a direct therapeutic to improve ASCVD, nonatherosclerotic CVD, and non-CVD is also worthy of additional investigation. The challenge will be to identify the specific subpopulations and disease states where HDL-related therapies are likely to have the most benefit and least harm. Preclinical data and observational data in humans support continued investigation of apoA-I as well as other mediators of HDL function in the development of new therapies for treating ASCVD, all other CVD, as well as non-CVDs in which HDLs and apoA-I are known to participate.

HDL THERAPEUTICS

The rationale for developing therapeutic agents that modulate HDL metabolism is based largely on 3 considerations. First, even after the introduction of more effective

LDL-C–lowering therapies, such as PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors, there will likely still be substantial residual ASCVD risk. Statins by themselves in the landmark clinical trials that led to their approval reduced ASCVD events only by approximately one-third. This has generated interest going back 2 decades in identifying other lipoprotein targets besides LDLs for drug development. The second factor is the consistent and long-standing observation of increased ASCVD events in participants with low HDL-C in large-scale epidemiologic studies.[94] Besides total cholesterol, the only other lipid measure used in most ASCVD risk equations is HDL-C. This association, however, as noted earlier, does not necessarily indicate that HDL-C is causally related to the pathogenesis of atherosclerosis. There is nevertheless relatively good biological plausibility for how HDL can be mechanistically related to atheroprotection, which is the third factor that has prompted efforts to develop HDL-based drugs. Numerous experimental studies show that HDLs have several potential antiatherogenic functions,[94] such as an ability to accept cholesterol that effluxes from cells, thereby stimulating reverse cholesterol transport.[5] In composition, HDLs are more diverse than LDLs and transport a large number of proteins and lipids and even miRNAs that may mediate various functions.[94] Preclinical animal models have also supported a direct role for HDLs in both blocking the progression of atherosclerosis and in promoting its regression,[95] but results from animal models do not always translate to humans.

Despite the promise of HDLs as a target for new cardiovascular drugs, the results so far have been disappointing. Recent genome-wide association studies and Mendelian randomization studies have questioned whether HDLs are causally related to atherosclerosis (as discussed previously). An alternative plausible explanation for its inverse association with ASCVD is that HDL-C is a marker for triglyceride-rich lipoproteins, which unlike HDL-C have clearly been implicated in genetic studies to cause ASCVD.[96] Increased triglycerides in triglyceride-rich lipoprotein particles are transferred to HDL by CETP. Subsequent lipolysis of HDL-associated triglycerides generates small HDL particles with less cholesterol.[97] This process of CETP-mediated lipid exchange also accelerates the catabolism of HDLs, which, along with the reduction in size, leads to an inverse association between HDL-C and triglycerides. HDL-C is a better ASCVD risk marker than triglycerides, but this may be attributable to its lower biological variability, allowing it to serve as a more stable marker of triglyceride metabolism.[94]

Most efforts to date have used HDL-C as the main metric for monitoring the effect of new drugs on HDL metabolism. We now know that some of the functional properties of HDLs may be better predictors of their atheroprotective properties than HDL-C. This assumption may have hindered our efforts so far to develop effective HDL-based drugs. The most persuasive examples of the importance of HDL function are the numerous studies showing that the cholesterol efflux capacity of HDLs is a better negative ASCVD risk factor than HDL-C.[5] From an evolutionary perspective, it has been suggested that a predominantly anti-atherosclerotic role for HDLs was unlikely because this condition is largely a disease of modern society and even today it frequently does not become clinically manifest until after childbearing age. It may be that one or more of the other pathophysiologic processes that HDLs modulate, such as inflammation,[8] are more relevant to its "true" biological role. Given, however, the pleiotropic structural role of cholesterol in cell membranes that affects many biological processes, including inflammation,[8] the connection between the cholesterol efflux capacity of HDL and atherosclerosis may still be relevant for developing HDL-based drugs.

In Table 3, the various types of drugs that raise HDL that have been approved or are being developed are described.[98] One of the first ASCVD drugs used is niacin. It raises HDL-C by 30% to 50% and lowers triglycerides and lipoprotein(a).[99] Two large randomized clinical trials (AIM-HIGH [Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglyceride: Impact on Global Health Outcomes] and HSP2Thrive [Heart Protection Study 2: Treatment of HDL to Reduce the Incidence of Vascular Events]) on niacin when used on top of statins in patients with relatively well-controlled LDL-C have failed to show any benefit in reducing clinical events despite increasing HDL-C. Whether niacin may be useful in other clinical settings, such as for patients with hypertriglyceridemia or with elevated lipoprotein(a), is not known. Fibrates, another old class of drugs, can also raise HDL-C by acting as peroxisome proliferator-activated receptor agonists, but they also have many other potential beneficial effects, particularly in lowering triglyceride-rich lipoprotein particles. Randomized clinical trials of fibrates on top of statins have failed to show benefit, but several ongoing studies are examining their possible usefulness in patients with elevated triglycerides. If these clinical trials are successful, it will likely not be clear whether modulation of HDL-C played a role.

CETP inhibitors were the first drugs specifically designed for increasing HDL-C. By blocking the equilibration of cholesteryl esters and triglycerides between the various lipoprotein classes, which is the main function of CETP, HDL-C is increased.[97] Several CETP inhibitors have been investigated, but they have mostly failed to show ASCVD benefit from raising HDL-C in clinical trials when used in conjunction with statins.[100–102] Anacetrapib modestly lowered ASCVD events in a large phase III study, but this was attributed to its ability to also lower LDL-C.[103] This is consistent with a recent Mendelian randomization study revealing that genetic variants with decreased CETP function are associated with lower

Table 3. Drugs Affecting HDL Metabolism

Drugs	HDL cholesterol elevation, %	Mechanism of action	Stage of development
Niacin	30 to 50	Multifactorial effects	Approved
Fibrates (fenofibrate)	5 to 15	Multifactorial effects by acting as PPAR agonists	Approved
CETP inhibitors (anacetrapib, dalcetrapib)	25 to 135	Inhibit transfer of cholesteryl esters away from HDL	Phase III (completed)
BET inhibitor (apabetalone)	5 to 10	Epigenetic modification altering apoA-I transcription	Phase III (completed)
HDL infusion therapy (CSL-112)	50 to 200	Direct infusion of exogenous HDL	Ongoing phase III (AEGIS-II)
Recombinant LCAT (MEDI6012)	50 to 100	Increase esterification of cholesterol on HDL	Ongoing phase II (REAL-TIMI 63B)

AEGIS-II indicates Study to Investigate CSL112 in Subjects With Acute Coronary Syndrome; apoA-I, apolipoprotein A-I; BET, bromodomain and extraterminal; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; PPAR, peroxisome proliferator-activated receptor; and REAL-TIMI 63B, A Study to Evaluate the Safety and Efficacy of MEDI6012 in Acute ST Elevation Myocardial Infarction.

apoB and LDL-C levels and decreased ASCVD events.[104] Dalcetrapib also failed to show benefit in a large phase III clinical trial,[102] but it is still being investigated, because in post hoc analysis patients with a relatively common *ADCY9* (adenylyl cyclase type 9) genotype may have had fewer clinical events on drug.[105] In a recent study of anacetrapib, however, there was no benefit in participants with the potentially beneficial *ADCY9* genotype.[106] Considering the recent progress using other nonstatin drugs for lowering LDL-C, and in light of numerous clinical trials demonstrating that raising HDL-C with CETP inhibitors does not contribute to ASCVD event reduction, there does not appear to be a role for the combined use of CETP inhibitors with statins, although the potential clinical usefulness of anacetrapib is not entirely known.

RVX-208 (apabetalone) represents another class of drugs that raises HDL-C modestly by epigenetically inhibiting the BET (bromodomain and extraterminal) family of proteins.[107] BETs are a large class of proteins that recognize histone acetylation and have wide-ranging effects on gene expression. They have been studied mostly in an oncogenesis setting. Their wide-ranging effects make it challenging to develop drugs on the basis of this target, but the possibility exists that they may have multiple beneficial effects. In fact, RVX-208 not only raises HDL-C but also decreases C-reactive protein and improves insulin sensitivity.[107,108] In phase II trials, mixed results were found,[108] and in a recent phase III trial (BETonMACE [Effect of RVX000222 on Time to Major Adverse Cardiovascular Events in High-Risk T2DM Subjects With CAD]) in patients with acute coronary syndrome and type 2 diabetes[109] RVX-208 did not reduce clinical events compared with placebo.

Promising animal studies showing that a small number of intravenous infusions of HDLs can rapidly reverse atherosclerotic plaque[95] have stimulated efforts by several drug companies to test rHDLs as a therapy. Either purified or recombinant apoA-I is combined with phospholipids in these different rHDL preparations. The rationale for acute therapy is that once a patient presents with a myocardial infarction, he or she is at high risk of having a second event, but it typically takes >1 year to see a significant reduction in clinical events after beginning statin treatment. The expectation is that acute treatment with rHDLs over several weeks and concurrently starting statin therapy could rapidly stabilize patients. The early-stage clinical trials that were based largely on intravascular ultrasound imaging of plaque in coronary vessels were encouraging,[95] but later larger phase II clinical trials also based on intravascular ultrasound imaging did not show evidence for significant improvement.[110] One formulation of rHDL that uses apoA-I purified from plasma called CSL112[111] is under evaluation for cardiovascular outcomes in a phase III clinical trial of patients with acute coronary syndrome (AEGIS-II [ApoA-I Event Reducing in Ischemic Syndrome II]). Small synthetic apoA-I mimetic peptides that share many of the same biological functions of apoA-I but have several potential advantages in terms of drug development have also been used alone or complexed with lipid in rHDL.[112] These apolipoprotein mimetic peptides have also been tested in early-stage clinical trials mostly for safety, but their development will likely depend on the success of CSL112.

Another strategy for raising HDL-C involves treatment with recombinant LCAT, which esterifies cholesterol in HDLs and to a lesser degree in LDLs. Patients with a genetic defect in LCAT have familial LCAT deficiency and present with low HDL-C, corneal opacities from cholesterol deposition, and anemia. Their main clinical problem is renal failure attributable to deposition of lipoprotein X, an abnormal multilamellar vesicle rich in phospholipid and free cholesterol.[113] Despite their low HDL-C, patients with familial LCAT deficiency do not appear to have increased atherosclerosis on the basis of carotid intima media thickness studies,[114] possibly because they also have low LDL-C. Nevertheless, recombinant LCAT is being considered as an

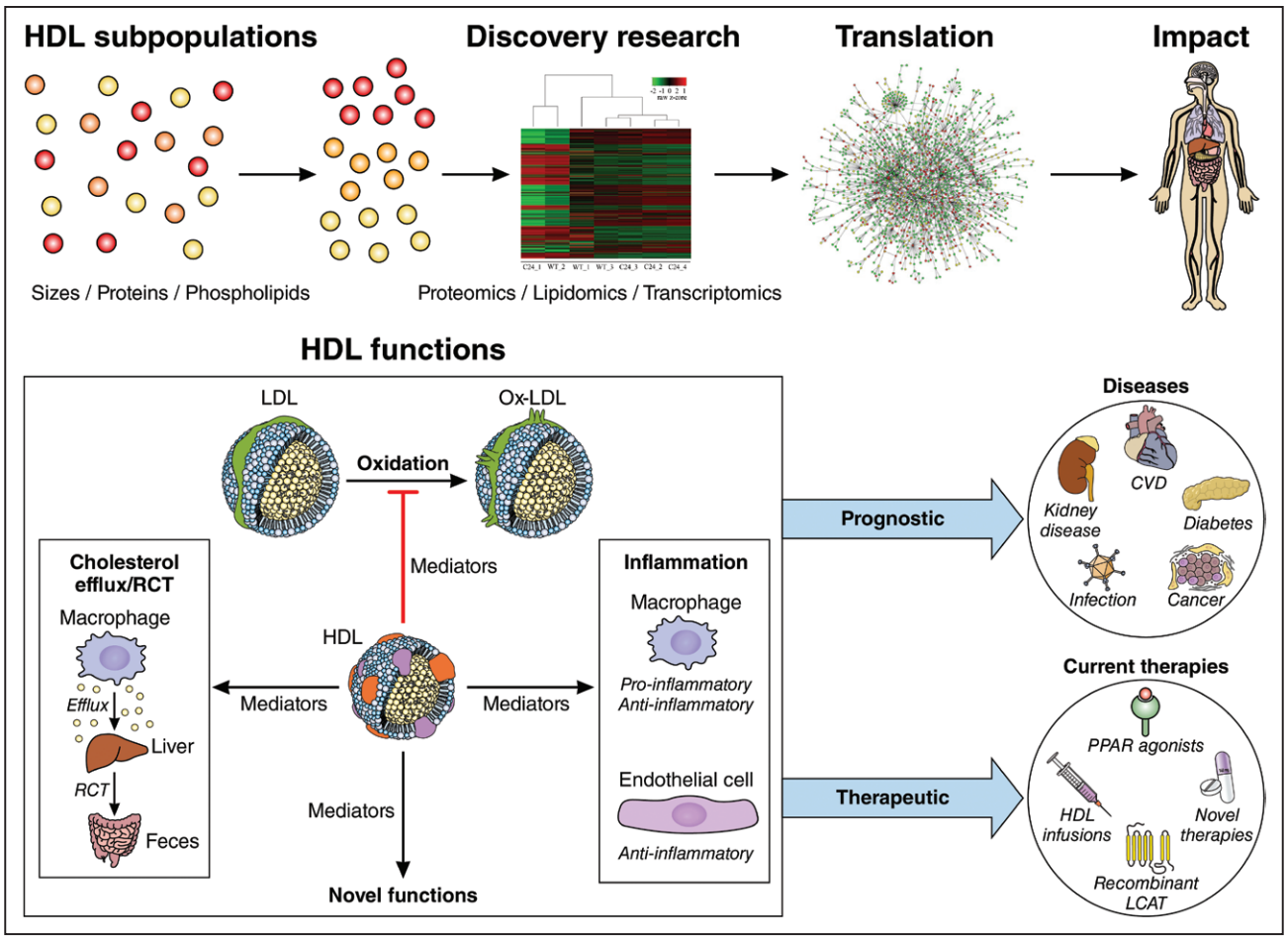

Figure 3. A conceptual framework for investigating the translational and clinical effects of HDLs.
HDLs comprise multiple subpopulations with diverse functions that are context dependent. Use of -omics approaches in diverse human cohorts with and without disease will help identify these context-dependent functions of specific HDLs to improve risk prediction and therapeutic strategies for both cardiovascular and noncardiovascular diseases. CVD indicates cardiovascular disease; HDL, high-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; PPAR, peroxisome proliferator-activated receptor; and RCT, reverse cholesterol transport.

intravenous infusion drug therapy to raise HDL-C for the acute treatment of patients with myocardial infarction. In a phase I study, an early formulation of recombinant LCAT was shown to be safe and raised HDL-C by ≈50% for a week.[115] A new formulation (MEDI6012) of recombinant LCAT with better pharmacokinetic and pharmacodynamic parameters is being tested on infarct size in a phase II study in patients with acute coronary syndrome (REAL-TIMI 63B [A Study to Evaluate the Safety and Efficacy of MEDI6012 in Acute ST Elevation Myocardial Infarction]). Recombinant LCAT may also be a useful therapy for preventing renal disease in patients with familial LCAT deficiency and was shown to nearly correct the abnormal lipoprotein profile in 1 patient with familial LCAT deficiency.[116] There are also early efforts for activating LCAT with small molecules,[117] which may be a more attractive approach that could be developed into a chronic therapy, if the early studies on recombinant LCAT turn out to be successful.

Several different types of drugs for modulating HDL have been tested for preventing and treating ASCVD, but none has proven to be successful. It may be that HDLs are not causally related to ASCVD, making these efforts futile, or the effect of HDLs in the face of effective LDL-C lowering may be too minimal to be clinically meaningful. More research related to HDL function may uncover other metrics of HDLs besides HDL-C that are causally linked to the development of atherosclerosis, enabling the development of successful HDL-based drugs for ASCVD or other disorders.

CONCLUSIONS

This article summarizes critical aspects of HDL structure, function, and metabolism. Implications for clinical usefulness will likely hinge on several key points: clarifying the dynamic and context-dependent nature of HDL function in multiple disease states; improved understanding of the regulation of HDL function at the molecular level; extending and further validating -omics analyses of HDL-associated proteins, lipids, and other constituents;

specifying CVD phenotype, acute versus chronic background illness, and concurrent medical therapy in human studies; and focusing on therapeutic strategies that optimize HDL functions in the right patients at the optimal time in their disease course. We recommend moving away from HDL-C levels as a focus of investigation. Instead, we propose that increased use of deep phenotyping approaches (-omics) in diverse populations will reveal specific HDL subpopulations that convey specific functions. Investigation of multiple functions within the same populations will likely yield metabolic networks that more precisely elucidate the role of HDLs in CVD as well as cancer, infection, diabetes, renal disease, and other disorders. Determining the role of inflammation and reverse cholesterol transport on these HDL function–disease relationships will be critical for translation of HDLs as diagnostic or therapeutic targets (Figure 3).

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