Abstract  For decades, HDL and HDL-cholesterol (HDL-C) levels were viewed as synonymous, and modulation of HDL-C levels by drug therapy held great promise for the prevention and treatment of cardiovascular disease. Nevertheless, recent failures of drugs that raise HDL-C to reduce cardiovascular risk and the now greater understanding of the complexity of HDL composition and biology have prompted researchers in the field to redefine HDL. As such, the focus of HDL has now started to shift away from a cholesterol-centric view toward HDL particle number, subclasses, and other alternative metrics of HDL. Many of the recently discovered functions of HDL are, in fact, not strictly conferred by its ability to promote cholesterol flux but by the other molecules it transports, including a diverse set of proteins, small RNAs, hormones, carotenoids, vitamins, and bioactive lipids. Based on HDL's ability to interact with almost all cells and deliver fat-soluble cargo, HDL has the remarkable capacity to affect a wide variety of endocrine-like systems. In this review, we characterize HDL's unique cargo and address the functional relevance and consequences of HDL transport and delivery of noncholesterol molecules to recipient cells and tissues. —Vickers, K. C., and A. T. Remaley. HDL and cholesterol: life after the divorce? J. Lipid Res. 2014. 55: 4–12.

Supplementary key words microRNA • extracellular miRNA • small RNA carrier

Over a half century of research has largely defined HDLs by their ability to accept free cholesterol from cells and transport cholesteryl esters (CEs) to recipient cells and tissues, including the liver where cholesterol is processed for excretion through bile salt conversion or direct secretion into the bile. HDL and HDL-cholesterol (HDL-C) levels are often considered to be one and the same and referred to as “good” cholesterol, mainly due to the epidemiologically observed inverse association of HDL-C to cardiovascular disease risk. As a consequence, billions of dollars and herculean effort were invested into HDL biology and pharmacological strategies to raise HDL-C levels for the prevention and treatment cardiovascular disease. Although epidemiological studies clearly defined a link between HDL-C levels and cardiovascular disease risk, recent clinical trials aimed at raising HDL-C levels have either been terminated early because of futility or even led to negative results in terms of causing an increase in cardiovascular events (1, 2).

One of the first studies aimed at increasing HDL-C levels was the VA-HIT, which used the fibrate drug gemfibrozil (1). Largely successful, this study provided “proof-of-concept” for subsequent studies. Unfortunately, later larger studies have so far failed to observe the predicted decrease in cardiovascular events associated with raising HDL-C levels. Niacin, one of the most effective drugs for raising HDL-C, showed lack of efficacy in the AIM-HIGH trial, although this has been attributed to the trial design (2, 3). Likewise, the HPS2-THRIVE found that extended-release niacin with laropiprant, a DP1 antagonist to reduce flushing, did not improve cardiovascular health or decrease cardiovascular events compared with statin-only therapy (mercknewsroom.com). The HDL field is also anxiously awaiting results from multiple clinical trials aimed at raising HDL-C levels through inhibition of cholesteryl ester transfer protein (CETP), a lipoprotein-associated protein responsible for the transfer of CEs from HDL to LDL. Torcetrapib and dalcetrapib, both CETP inhibitors, have recently failed to reduce cardiovascular outcomes in large clinical trials and, in some cases, increased cardiovascular events compared with taking statins alone (4). Torcetrapib was found to increase, not decrease, cardiovascular events in the ILLUMINATE trial; however, these observations are likely the result of off-target effects (5). Dalcetrapib advanced to phase III clinical trials in a study to evaluate its...
efficacy in reducing cardiovascular outcomes, dal-OUTCOMES (6). Two successful earlier studies concluded that dalcetrapib raised HDL-C levels and decreased vascular inflammation in the dal-VESSEL and dal-PLAQUE trails, respectively. Nevertheless, the much larger dal-OUTCOMES study was terminated early based on futility, the inability of dalcetrapib to reduce adverse cardiovascular events even though HDL-C levels were found to be increased by 30% (7). Evacetrapib and anacetrapib, both next-generation CETP inhibitors, are currently in large phase III clinical trials to assess cardiovascular outcomes associated with increasing HDL-C levels. Results from these trials are eagerly anticipated as both evacetrapib and anacetrapib have been found to increase HDL-C levels over 125%, which is a substantial advancement from dalcetrapib and other CETP inhibitors (8–11). As such, the HDL field remains cautiously hopeful in CETP inhibition as a means to increase HDL-C levels and reduce cardiovascular outcomes. In addition, the HDL-C hypothesis has taken another hit in the area of genomics, as genetic polymorphisms that are associated with increased HDL-C levels were recently found to have no benefit to cardiovascular risk or events (12).

Although its image has recently taken a publicity hit, HDL still remains a remarkable and highly beneficial factor in the multiple layers of systemic homeostatic control beyond cholesterol metabolism. Although it may be premature to suggest at this time, the noncholesterol cargo of HDL may ultimately be more closely linked to its beneficial anti-atherogenic properties and may confer many of HDL’s benefits outside of cardiovascular disease (Fig. 1). In support of this theory, HDL particle number has recently been found to be a better indicator of cardiovascular disease risk than HDL-C levels (13). Although this may be due to the increased ability of more HDL particles for removing excess cholesterol, increasing HDL particle numbers may also affect other functions of HDL and its ability to transport other cargo. Therapeutic strategies to increase HDL particle number may turn out to be better in reducing cardiovascular events than simply increasing HDL-C.

It should be noted that HDL efflux capacity from macrophages has also been found to be a better marker for cardiovascular disease risk than HDL-C levels (14). HDL’s ability to participate in the reverse cholesterol transport (RCT) pathway is, therefore, likely an important part of its anti-atherogenic role. We are now beginning to realize the full breadth of HDL’s function and cargo; thus, there is a significant need to begin to rethink and possibly focus on these other aspects of HDL besides its role in transporting cholesterol. Although it is not clear what the new focus on HDL should be, we review here the latest findings related to the metabolome, transcriptome, lipidome, and proteome of HDL.

**Table 1**

| Cargo Class | Examples |
|-------------|----------|
| Lipids      | Cholesterol, triglycerides, phospholipids |
| Steroids    | Testosterone, estradiol, cortisol |
| Vitamins    | A, D, E, K |
| Hormones    | Estrogen, testosterone, insulin |

**HDL METABOLOME**

Besides lipids, we know relatively little about the repertoire of small molecules circulating on HDL; however, this will likely be an emerging new field for HDL research. As HDL is possibly rebranded as a general transporter of cargo between cells, a comprehensive characterization of HDL’s metabolome is warranted. Specific examples of small molecules transported on HDL, which are primarily hydrophobic, are shown in Table 1.

Some of the best studied small metabolites carried by HDL are the fat-soluble vitamins (15–18). A majority of α-tocopherol (vitamin E), for example, is transported in plasma by lipoproteins, including HDL, and delivered to recipient cells by means of lipoproteins. Most interestingly, HDL delivery of vitamin E to epithelial cells was found to be independent of scavenger receptor BI (SR-BI), as chemical inhibition of with basic lipid transport-1 (BLT-1) failed to reduce vitamin E delivery (19). Conversely, endothelial cell uptake of HDL-vitamin E was found to be mediated by SR-BI (15, 20). Vitamin E is an essential antioxidant vitamin, although the relevance of HDL-mediated vitamin E transfer between cells is currently unknown. Vitamin E is also found on other lipoproteins, including chylomicrons, which have been found to deliver vitamin E to peripheral tissues (21); however, the delivery capacity and dependency of each lipoprotein class to different cell types is largely unknown.

Unlike vitamin E, most of vitamin D is believed to be bound to a specific serum-binding protein. The vitamin D-binding protein, however, is routinely found in proteomic studies to be associated with HDL (22, 23). Most interestingly, vitamin D and vitamin D receptor modulators have been found to inhibit apolipoprotein A-I (apoA-I) (24, 25). Although HDL-vitamin D transport and delivery has not been directly demonstrated, if HDL does indeed transfer vitamin D to the liver, it may participate in a feedback mechanism to govern HDL biogenesis. Retinol (vitamin A) is also likely transported in various forms on HDL. Similar to vitamin D, retinol associates with a binding protein, the plasma retinol-binding protein (PRBP), which has also been reported in multiple studies to be associated with HDL (23). Retinol, like cholesterol, is stored and transferred in its esterified form, retinyl ester, which can be transferred between HDL and LDL by CETP. Evidence suggests that retinol may associate with HDL both through its association with PRBP and direct incorporation into HDL lipid core after esterification. Retinol inside the cell is converted to biologically active retinoic acid, which acts as a transcriptional ligand for multiple nuclear receptors, such as retinoic acid receptors (RARs and RARB) and retinoid X receptors (RXRs, RXRβ, and RXRγ). These nuclear receptor transcription factors control the expression of many lipid-, cholesterol-, bone-, and metabolism-regulating genes; therefore, HDL transfer of retinol or retinyl ester to recipient cells may mediate the expression of many metabolic genes (26). Although plausible, experimental validation and verification is needed to demonstrate HDL transport and transfer of retinol or retinyl ester to recipient cells. Moreover, it remains to be determined whether HDL increases retinoic acids levels in treated cells or whether HDL induces RAR/RXR transcriptional activation.

In addition to fat-soluble vitamins, multiple studies have reported that HDL transports steroids and other hormones. In 1989, HDL and apoA-I were found to bind to the lipo-philic thyroid hormone thyroxine (T4) (27). A few years
| Factor Type | Component | Possible Function | Cell Type or Tissue | Ref. |
|-------------|-----------|------------------|---------------------|-----|
| Vitamin     | α-Tocopherol (vitamin E) | Essential anti-oxidant vitamin | Epithelial, EC | (15, 19, 20) |
| Steroid     | Thyroxine (T4) | Thyroid hormone, development, and metabolism | Many | (27, 28) |
| Hormone     | Estrogen | Nuclear receptor activation, steriod synthesis precursor | Ovary, adrenal, hepatocyte | (29) |
| Hormone     | Pregnenolone | Nuclear receptor activation, steriod synthesis precursor | Ovary, adrenal, hepatocyte | (29) |
| Hormone     | Dehydroepiandrosterone | Nuclear receptor activation, steriod synthesis precursor | Ovary, adrenal, hepatocyte | (29) |
| Bile acid   | Chenodeoxycholic acid | Signal activation, cytotoxicity, tumorigenesis | Peripheral cell | (39, 41) |
| Bile acid   | Cholic acid | Signal activation, cytotoxicity, tumorigenesis | Peripheral cell | (39, 42) |
| Bile acid   | Lithocholic acid | Signal activation, cytotoxicity, tumorigenesis | Peripheral cell | (39) |
| Bile acid   | Glycochenodeoxycholic acid | Signal activation, cytotoxicity, tumorigenesis | Peripheral cell | (39) |
| Carotenoid  | β-Carotene | Antioxidant, pigment for retina, healthy skin, immune system | Retina, macula, skin, bone | (18) |
| Carotenoid  | α-Carotene | Antioxidant, pigment for retina, healthy skin, immune system | Retina, macula, skin, bone | (18) |
| Carotenoid  | Lycopen | Antioxidant | Lung, stomach, prostate | (18) |
| Carotenoid  | Lutein | Antioxidant, pigment for retina | Retina, macula | (18) |
| Carotenoid  | Cryptoxanthin | Antioxidant, tumor suppressor | Lung | (18) |
| Metabolite  | Ubiquinone | Prevents heart failure, cancer, migraines, and hypertension | Cardiomyocytes, neurons, vascular cell | (40) |
| Metabolite  | Heneicosanoic acid | Saturated fatty acid | Many | (47) |
| Metabolite  | Pentitol | Sugar metabolism | Fibroblast, erythrocyte | (47) |
| Metabolite  | Oxalic acid | Reducing agent, chelator | Many | (47) |
| Bioactive lipid | S1P | Repress adhesion molecule expression, VSMC migration, MCP-1 expression; stimulate eNOS, PI3K/ERK, RhoK, and p38 MAPK | EC, VSMC | (53) |
| Bioactive lipid | Lysoxosulfatide | Stimulate eNOS, activate Akt, suppression of apoptosis | EC | (51) |
| Bioactive lipid | Sphingosylphosphorylcholine | Activate Akt, suppression of apoptosis | EC | (51) |
| Protein     | ApoA-I | Repress adhesion molecule expression, increase UCP1 mRNA levels, activate STAT3, repress cytokine expression | EC, adipocyte, macrophage | (60) |
| Protein     | Hemoglobin (hb), haptoglobin (hp), hemopexin (hx) | Pro-inflamatory | Neuron, brain, many | (86) |
| Protein     | α1-antitrypsin | Prevents VSMC matrix degradation | VSMC/ECM | (83) |
| Protein     | PON1 | Inhibits HDL oxidation, required for HDL-mediated NO stimulation | EC, many | (84, 85) |
| RNA         | MicroRNA | Post-transcriptional gene regulation | Hepatocyte, many | (88) |
| RNA         | Rnase P-derived small RNA | Unknown regulatory function | Many | (88) |
| RNA         | tRF | Unknown regulatory function | Many | (88) |
| Binding protein | Vitamin D binding protein | Transport of vitamin D* | Many | (22, 23) |
| Binding protein | Retinol binding protein | Transport of retinol (vitamin A)* | Many | (22, 23) |
| Binding protein | Transthyretin | Transport of retinol (vitamin A)*, thyroxine (T4) | Many | (23) |
| Binding protein | Serotransferrin | Transport of Iron, iron homeostasis | Duodenum, macrophage | (70) |

EC, endothelial cell; VSMC, vascular smooth muscle cell.
*Predicted, but not directly demonstrated or experimentally determined.

later, T4 was found to bind to and interact with many apolipoproteins, including apoA-II, apoC-I, apoC-II, and apoC-III (28). More recent studies have identified transthyretin on HDL, which also strongly binds to both T4 and retinol (23). HDL has also been reported to transport esterified hormones, including estrogen (estrodial), pregnenolone, and dehydroepiandrosterone (29). At this time, the physiological roles of HDL-associated hormones is largely unknown; however, HDL has been found to delivery 17-estrodial to recipient hepatoma cells in part of a likely HDL-endocrine transport system. Circulating hormones (e.g., estrogen) can be esterified by lecithin-cholesterol acyltransferase (LCAT) on HDL and readily incorporated into HDL. Esterified HDL hormones can also be transferred to LDL through CETP activity. The delivery of esterified HDL hormones to recipient cells was found to be dependent upon SR-B1, as inhibition of SR-B1 with BLT-1 blocked HDL transfer (30). Once delivered to recipient cells, HDL hormones may possibly act as transcriptional regulators acting on nuclear receptor transcription factors or may serve as precursors for cellular steroid synthesis (31–33). HDL-delivered pregnenolone has been demonstrated to be a precursor for ovarian progestin and adrenal steroids (32, 33). Results from these early studies support a possible role of HDL as a major carrier and depot for hormones in the endocrine system (Table 1). Similar to other HDL cargo, the intercellular transfer of hormones by HDL reinforces its role as a diversified cellular messenger in systemic homeostasis.

In peripheral tissues, bile acids (BA) activate numerous signaling cascades, induce tumorigenesis, and may be
affinity to BAs than albumin (35). Both conjugated and BAs are elevated, HDL has been found to have a greater injury or cholestasis when concentrations of circulating also found in lipoprotein fractions. Strikingly, during liver also found in lipoprotein fractions. Strikingly, during liver injury or cholestasis when concentrations of circulating BAs are elevated, HDL has been found to have a greater affinity to BAs than albumin (35). Both conjugated and unconjugated BAs have been reported to bind to HDL; however, BAs are also found in lipoprotein fractions. Strikingly, during liver injury or cholestasis when concentrations of circulating BAs are elevated, HDL has been found to have a greater affinity to BAs than albumin (35). Both conjugated and unconjugated BAs have been reported to bind to HDL; however, conjugated BAs are overrepresented on HDL (39). The position of the 3α-hydroxyl group in the steroid nucleus, specifically in cholate and taurocholate derivatives, was found to promote HDL binding (40), particularly the more hydrophilic BAs. In acute cholestatic patients, SCARB1 heterozygous carotene, has been found to inhibit atherosclerosis in the eye, and thus, HDL delivery of carotenoids to the eye serves to protect against macular degeneration, cataracts, and blindness. β-cryptoxanthin, another carotenoid, has been reported to suppress cell proliferation and inhibit lung cancer; therefore, HDL transfer and delivery of cryptoxanthin could possibly antagonize tumorigenesis (44). One of the most widely studied carotenoid, β-carotene, has been found to inhibit atherosclerosis in hypercholesterolemic rabbits and is associated with HDL (45). HDL has also been found to transport ubiquinone (UQ, coenzyme Q or coenzyme Q₁₀) and may facilitate the intercellular transfer of UQ or dietary intake delivery of UQ to recipient cells (46). Although the role of UQ in disease is not fully understood, HDL-mediated transport of UQ has been proposed to possibly help prevent heart failure, cancer, migraines, and hypertension.

Although a majority of HDL’s small molecules are likely to be nonpolar or hydrophobic, a recent study identified numerous polar metabolites on HDL, including heneicosanoic acid, pentitol, and oxalic acid, which were found to be significantly correlated with insulin resistance (HOMA-IR) (47). HDL transport of small molecules and bioactive metabolites in Table 1 illustrates the diversity of biological pathways that HDL can possibly regulate. Currently, the functional relevance of the intercellular transfer of the HDL metabolome is not fully understood, nor have we likely identified all the small molecules that can be transported by HDL. A comprehensive analysis of the HDL metabolome is warranted and will likely uncover other metabolites and homeostatic networks affected by HDL.

**HDL LIPIDOME**

The lipids carried by HDL can be largely categorized as being either neutral hydrophobic lipids (cholesterol esters and triglycerides), which are carried in the core of HDL, or amphipathic lipids, such as cholesterol or phospholipids, which are on the surface of HDL. In the classic view of HDL metabolism, the core lipids are the cargo that is being transported, whereas the surface lipids are simply components of the transport vehicle. Recently, it has been realized that some of the lipids carried by HDL can be transformed into potent bioactive molecules. For example, phosphotidylcholine (PC), the most common phospholipid on HDL, is highly susceptible to oxidation, which after hydrolysis generates the highly reactive and damaging lyso-phosphotidylcholine (LPC) (48, 49).

Sphingomyelin (SM), another common class of lipids on HDL, is a substrate for nSMase2 in the ceramide-signaling pathway (50). SM can also be converted to lysosphingolipids, namely, sphingosylphosphorylcholine and lysosulfatide, which have been found to protect against endothelial apoptosis through activation of Akt signaling (51). Most interestingly, the Akt-signaling cascade is responsible for HDL-lipid suppression of apoptosis, as inhibition of Akt phosphorylation of Bcl-2-associated death (BAD) promoter blocked the HDL-lipid effect (51). HDL has been shown to have several beneficial effects on endothelial cells, which is possibly mediated by HDL lipids (52). HDL protection of vascular endothelium is two-fold: HDL inhibits apoptosis and enhances cell survival signaling. In addition, HDL transports the bioactive lysosphospholipid sphingosine-1-phosphate (SIP), which promotes endothelial survival (53). Likewise, HDL SIP has been found to repress the expression of endothelial adhesion molecules,
HDL PROTEOME

The major HDL protein is apoA-I, which accounts for approximately 70% of total HDL protein. ApoA-I serves as the main structural protein on HDL (60), but it can also contribute to the ability of HDL to efflux cholesterol from cells by interacting with the ATP-binding cassette transporter A1 (ABCA1) (61). Recent findings, however, suggest that its role may be not limited to these two functions. Over 15 years ago, apoA-I was found to block oxidized LDL (oxLDL)-mediated endothelial cell calcium burst and apoptosis through initiating gene expression. This effect was found to be dependent upon protein synthesis and direct interaction of apoA-I and endothelial cells (62).

Jack Oram and colleagues found that HDL stimulates the STAT3-signaling cascade through apoA-I activation of ABCA1 in macrophages, ultimately blocking lipopolysaccharide (LPS) induction of cytokines (63). The key observation was that HDL controlled gene expression in cells it interacts with outside of cholesterol flux. In rabbits, HDL was found to suppress endothelial expression of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), likely through apoA-I, as glycation of apoA-I attenuates HDL’s anti-inflammatory effect (64, 65). ApoA-I was also found to mediate HDL’s ability to inhibit integrin CD11β expression in monocytes and neutrophils, which likely contributes to reduced vascular inflammation and atherosclerosis (66, 67). Similarly, apoA-I was found to inhibit monocyte differentiation to dendritic cells through IL-10 and PGE2 induction (68).

ApoA-I was also found to increase uncoupling protein 1 (UCP1) mRNA in adipose (brown) in vivo (69). Understanding the mechanism behind these various effects of apoA-I is challenging, because it is difficult to differentiate a direct cellular effect of apoA-I on a cell-signaling pathway as opposed to an indirect effect from the cellular response from cholesterol efflux. For example, apoA-I-mediated decrease of CD11β expression in mononuclear cells is replicated by methyl-β-cyclodextrin, suggesting that cholesterol efflux from the plasma membrane is primarily responsible for gene regulation, not apoA-I-induced signaling. Many of apoA-I-attributed functions are most likely the cellular response to cholesterol efflux but perhaps not all, and this is an important area of future investigation. Besides apoA-I, it has been known for many years that HDL transports less-abundant apolipoproteins, such as apoA-II and apoE, but it was not until recently that the full repertoire of HDL proteins was appreciated (70).

An early clue to the complexity of the HDL proteome came from an analysis of its peptides, which found over 100 distinct peptide fragments carried on HDL from a wide variety of extracellular and intracellular proteins (71). In 2007, the first comprehensive analysis of the HDL proteome by Jay Heinecke’s laboratory found approximately 40 different HDL-associated proteins by shotgun proteomics that were loosely organized into four biological processes, namely lipid metabolism, acute-phase response, protease inhibitor, and complement regulation (22). Currently, over 100 unique proteins have been reported on HDL by mass spectrometry, including proteins that are involved in a diverse set of pathways, such as extracellular domains, antioxidants, immune responses, lipid binding, transport, and general binding (23). As such, HDL is now appropriately characterized as a circulating scaffold of loosely associated proteins, some of which may have specific effects on cardiovascular disease. The HDL proteome has been found to be highly malleable to disease, such as rheumatoid arthritis (72), uremia (73), endotoxemia (74), acute sex steroid withdrawal (75), acute coronary syndrome (23), and general cardiovascular disease (76–78).

Likewise, the HDL proteome has been found to respond to drug treatments and therapies, including hemodialysis (79), testosterone (80), omega-3 fatty acids (81), niacin (82), and statin therapies (82). Many proteins related to HDL’s anti-inflammatory properties are consistently found on HDL in all studies, including multiple complement activation proteins and proteolysis enzymes. Next to apolipoproteins, α1-antitrypsin is one of the most abundant proteins on HDL, and in tandem with HDL’s anti-elastase capacity, it serves to prevent extracellular matrix degradation associated with vascular smooth muscle cells and is inversely associated with aortic aneurysms (83).

One of the more widely studied HDL functions outside of RCT is HDL’s antioxidant capacity, which may confer many of HDL benefits across multiple pathophysiologicals. HDL’s antioxidant capacity is largely mediated by paraoxonase 1 (PON1), which has been found to be decreased on HDL from patients with cardiovascular disease. Likewise, HDL-PON1 levels have also been found to be altered in disease, including uremia, diabetes, and other cardiovascular diseases. PON1 activity is essential to protect HDL lipids from oxidation and apoA-I malondialdehyde formation (84). Furthermore, PON1 is required on HDL to activate eNOS and stimulate NO production in endothelial cells, as HDL from PON1-deficient mice failed to stimulate endothelial NO production (85).

Another interesting set of proteins transported by HDL are hemoglobin and its related binding proteins. Both
mouse and human HDL have been reported to transport haptoglobin (Hp), hemoglobin (Hb), and hemopexin (Hx), which were found to be increased with coronary heart disease in humans (86). Hp is required for HDL-Hb association, and HDL becomes highly inflammatory when it transports both Hp and Hb (86). Historically, Hb is known for its oxygen-carrying capacity, but it is also found in other cell types outside of red blood cells in which it serves as an iron-binding protein and antioxidant (87). When associated with HDL, Hb also likely acts as an antioxidant and may mediate HDL transport of iron. Hb is not the only iron-binding protein on HDL, as serotransferrin is also highly abundant on HDL and was recently found to be decreased on HDL after hemodialysis in patients with end-stage renal disease (79). HDL likely participates in the transport of iron from the gut and phagocytic cells to targeted recipient cells through serotransferrin and thus contributes to global homeostasis and cellular metabolism and physiology. The recent studies of the HDL proteome have revealed that besides the “classic” proteins on HDL, such as apoA-I, that largely serve a structural role in HDL or a role in its metabolism, HDL cargo includes other proteins that may have distinct biological effects and could account for some of the other functions of HDL besides cholesterol transport.

**HDL TRANSCRIPTOME**

Recently, we discovered that HDL also transports small RNAs, including microRNAs (miRNA), tRNA-derived RNA fragments (tRF), and RNase P-derived RNA fragments (88). The majority of RNAs on HDL are short in length (<70 nt) and single-stranded (88). Whether these small RNAs are associated with HDL lipids or possibly a binding protein is not known. The physiologic relevance of HDL small RNAs is also not clear; however, early work suggests that they can potentially serve as biomarkers of disease and can be delivered to cells and alter gene expression similar to what has been reported for exosomes and microvesicles (89).

HDL from healthy human subjects and mouse HDL were observed to possess a similar miRNA profile, and HDL miRNAs from both species were found to be significantly altered by hypercholesterolemia and atherosclerosis (88). HDL from wild-type c57Bl/6 mice on normal chow diet and apolipoprotein E-null (ApoE<sup>-/-</sup>) mice on chow or high-fat diet each had a distinct miRNA profile, suggesting that genetic dyslipidemia and diet-induced atherosclerosis uniquely alter HDL miRNAs. In humans, approximately 22 miRNAs were found to be significantly altered on HDL from subjects with familial hypercholesterolemia (FH), as quantified by TaqMan-based low-density arrays. Most interestingly, HDLs from FH subjects were found to significantly alter gene expression in cultured Huh7 hepatoma cells compared with HDL from normal subjects (88). Similar to other HDL-mediated cargo delivery, HDL transfer of miRNAs was found to be dependent upon SR-BI, as siRNA knockdown of SR-BI significantly inhibited HDL’s ability to deliver miRNAs to recipient cells. Furthermore, induction of human SR-BI in baby hamster kidney cells, which have minimal or no SR-BI expression, facilitated HDL transfer of functional miRNAs to cultured cells. HDL-mediated miRNA delivery was shown to affect the expression of luciferase reporter constructs containing the 3′ untranslated region of a putative mRNA target for the transferred miRNA (88).

In addition to miRNAs, other small RNAs are also present on HDL. miR-685 is a small RNA derived from RNase P and was found to circulate with mouse HDL (88). HDL miR-685 was upregulated (13.78-fold; P = 0.09) with hypercholesterolemia in ApoE<sup>-/-</sup> mice on a high-fat diet compared with c57Bl/6 wild-type mice on a chow diet (88). Currently, it is not known whether RNase P-derived fragments are found on human HDL or whether they are transferred to recipient cells and repress gene expression. In addition to small RNAs generated from RNase P, small RNA-like RNAs can be processed from small nucleolar RNA (snoRNA), small nuclear RNA or U-RNA (snRNA), tRNA, and tRF (90, 91). These newly discovered small RNAs represent an entirely new set of possible regulatory molecules whose physiological relevance is not currently understood; however, tRFs have been found to participate in gene silencing and are consistently observed on HDL (88, 92). Similar to miRNAs, tRFs have their own numerical nomenclature (e.g., tRF-1001); however, a few tRFs were initially identified as miRNAs (miR-1274A, miR-1274B, miR-1308, miR-866-5p, miR-1280, and miR-720) (90, 93). Using the TaqMan real-time PCR low-density array platform to quantify miRNAs on mouse HDL, miR-720 was found to be upregulated 8.75-fold in a mouse model of atherosclerosis, ApoE<sup>-/-</sup> mice on a high-fat diet compared with wild-type c57Bl/6 mice on a chow diet (88). miR-720 is predicted to be processed from tRNA<sup>Thr</sup> and, like other tRFs, is transcriptionally regulated by Ski-related novel gene (SnoN/SKIL) (94). SnoN knockdown was found to decrease miR-720, increase the putative miR-720 target p63, and alter cell proliferation in esophageal cancer cells (94). Therefore, if HDL transfers functional miR-720 to recipient cells, HDL may influence cell proliferation and tumorigenesis through the HDL-miRNA endocrine-like system.
Although HDL miRNAs hold great promise as biomarkers and likely serve as cellular messengers, extensive investigation into cellular selection, export, transport, delivery, and utilization is required. Most importantly, the functional relevance and physiological impact of extracellular miRNAs, particularly HDL miRNAs, needs to be demonstrated in vivo. The development and application of novel tools and in vivo reporter systems will be required to accomplish this feat.

SUMMARY

HDL is remarkable in that it interacts with many if not all organs, tissues, and cell types, which makes HDL a perfect transporter of cargo and cellular messages in intercellular communication. Here we detailed many of HDL’s less studied cargo, including vitamins, hormones, carotenoids, steroids, miRNAs, tRFs, lysosphingolipids, and loosely associated proteins. Recent studies have uncovered numerous novel functions of HDL, in which these other cargoes of HDL may play a significant role (Fig. 1). HDL, specifically small-sized HDL, has multiple anti-inflammatory properties, including repression of inflammatory signaling, adhesion molecule expression, and cellular activation, which are conferred by HDL proteins, lipids, and likely miRNAs (95). Likewise, many of HDL’s proteins, namely apoA-I and PON1, protect HDL particles and cells from oxidative damage. Outside of cholesterol efflux, HDL has many other beneficial functions, and unique miRNA, lipid, protein, and small molecule components likely mediate each, if not all of these functions, including cytotoxic, anti-atherosclerotic, anti-inflammatory, vasodilatory, and many other functions to be discovered. As such, HDL control of systemic homeostasis likely extends beyond control of only plasma or cellular cholesterol levels and likely includes the essential transfer of these described proteins, bioactive lipids, and miRNA in cell-to-cell communication and metabolism. It may be time to rethink the HDL cholesterol hypothesis, not necessarily to replace it, but to expand the role of HDL beyond that of cholesterol to incorporate some of the recent findings on the importance of the other cargo of HDL in cardiovascular disease.

PERSPECTIVE

There is a significant need to redefine HDL and its beneficial properties independent of cholesterol. HDLs transport a diverse set of functional proteins, including many binding proteins. HDLs transport and deliver vitamins, carotenoids, and other small molecules. Moreover, HDLs transport hormones, steroids, and bile acids and can modulate multiple endocrine pathways. HDLs also transport and deliver microRNAs to recipient cells and control gene expression. Likewise, HDLs carry bioactive lipids and can activate signaling cascades and receptors that control endothelial apoptosis, migration, survival, and activation. Many of HDL’s alternative non-cholesterol cargo likely confer many of HDL’s alternative functions.

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