Advances in high-density lipoprotein physiology: surprises, overturns, and promises

Caterina Constantinou,* Eleni A. Karavia,* Eva Xepapadaki,* Peristera-Ioanna Petropoulou,* Eugenia Papakosta, Marilena Karavryaki, Evangelia Zwintzou, Vassilis Theodoropoulos, Serafoula Filou, Aikaterini Hatziri, Christina Kalogeropoulou, George Panayiotakopoulos, and Kyriakos E. Kypreos
Pharmacology Department, University of Patras Medical School, Rio Achaias, Greece

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Since its initial discovery in 1929 by Michel Macheboeuf (139), high-density lipoprotein (HDL) has been an intriguing macromolecular assembly of proteins and lipids offering constant excitement and surprises to the biomedical community. In recent decades, HDL attracted a lot of attention mainly because of its important role in atheroprotection. However, more recent findings propose a multifunctional role of HDL in numerous other biological processes, including inflammation, oxidative stress and nitric oxide (NO) production, and regulation of plasma glucose homeostasis (84).

The inverse correlation between HDL cholesterol (HDL-C) levels and the risk for developing coronary heart disease (CHD), initially identified by pioneer lipidologists John Gofman (57) and Richard Havel (64) and later confirmed by the Framingham Heart Study (58) and numerous other clinical and epidemiological studies (84, 107, 132, 179), led to a principle that dominated lipidology from the 1950s till now: high HDL-C levels in plasma are protective against the development of atherosclerosis (84). Indeed, recent data show that HDL is a mixture of distinct lipoprotein particles that are heterogeneous in size as well as in lipid and apolipoprotein composition, with densities ranging between 1.063 to 1.210 g/ml (126). Mature spherical HDL contains 45–55% (as mass %) apoproteins, 26–32% phospholipids, 15–20% esterified cholesterol, 3–5% free cholesterol, and ~5% TG (84, 179). Variations in apolipoprotein content of these particles set the basis for functional heterogeneity of HDL between individuals (126), and differences in lipid composition may give HDL either a discoidal or spherical shape (84, 179).

HDL, obesity, type 2 diabetes mellitus, inflammation, bone, brain, obstructive pulmonary disease, and other metabolic disorders, such as obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, inflammation and sepsis, bone and obstructive pulmonary diseases, and brain disorders.

HDL particle biogenesis involves lipid transporters ATP-binding cassette A1 and G1 (ABCA1 and ABCG1) and the plasma enzymes lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP) (27, 53, 56, 88, 165). Studies in...
Experimental mouse models showed that in addition to apolipoprotein A-I (apoA-I), other apolipoproteins such as apoE (108) and apoC-III (106) may also promote the de novo biogenesis of HDL independently of preexisting classical apoA-I-containing HDL (84). The main lipid cargo of mature HDL particles is esterified cholesterol. Following its biogenesis in the periphery, HDL is catabolized via scavenger receptor class B member I (SR-BI) mainly in the liver, an 82-kDa cell-surface glycoprotein that belongs to the CD36 superfamily (102). The main function of SR-BI is the selective uptake of cholesteryl esters (CE) from HDL, thus reducing its size (14, 37, 171, 173), the final step of a process referred to as “reverse cholesterol transport”.

In addition to atherosclerosis and CHD, recent data indicate that HDL may play pivotal role in the pathology and treatment of other diseases, including morbid obesity, nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus, bone metabolic diseases, obstructive pulmonary disease, and numerous diseases of the central nervous system (summarized in Figs. 1 and 2). In the next paragraphs, we review this novel information and provide our own perspective on the future of HDL pharmacology and targeted therapeutics.

**Role of HDL in obesity and hepatic lipid deposition.** Body fat accumulation and hepatic lipid deposition associated with insulin resistance and glucose intolerance are closely associated with the development of metabolic syndrome (MetS). Subjects with MetS also have dyslipidemia, which is characterized by significantly elevated plasma TG levels and drastically reduced HDL-C levels. To investigate any potential relationship between obesity and HDL quality and functionality, initially we focused our attention to the relevant clinical example of rapid weight loss following bariatric surgery, associated with a reduced risk for CHD later on in life (147).

Analysis of HDL particle composition showed that rapid weight loss was associated with a significant switch from a primarily apoE- and apoC-III-containing HDL to a primarily apoA-I-containing HDL (210). This compositional change was associated with a significant improvement of the antioxidant properties of HDL. Concomitantly, we observed a significant improvement of the plasma enzymatic activity signature of LCAT and CETP (210). These data provided an initial indication that reduced apoA-I content of HDL correlates with less functional HDL in obese subjects (210).

Therefore, to determine if HDL in plasma has a causative role in the development of obesity and hepatic lipid deposition, we turned our attention back to the study of individual molecular partners of the HDL metabolic pathway in experimental mouse models. Through a series of experiments in ABCA1-deficient (abca1<sup>−/−</sup>), apoA-I-deficient (apoa1<sup>−/−</sup>), LCAT-deficient (lcat<sup>−/−</sup>), and SR-BI-deficient (scarb1<sup>−/−</sup>) mice, we discovered that, in addition to the classical role of HDL in plasma cholesterol metabolism, it also plays significant role in postprandial TG metabolism and clearance, associated with the obese phenotype.

Specifically, we found that lack of HDL in abca1<sup>−/−</sup> mice resulted in the accumulation of apoC-III and potentially other exchangeable apolipoproteins (such as apoE and apoA-I) primarily on TG-rich lipoproteins, leading to inhibition of lipoprotein lipase (LPL)-mediated lipolysis of their TG and the development of hypertriglyceridemia (106). In agreement with these data, adipose tissue-selective deletion of ABCA1 (ABCA1<sup>ad/ad</sup>) showed that it also regulates adipocyte lipid content and adipose tissue glucose tolerance and insulin sensitivity. Indeed, ABCA1<sup>ad/ad</sup> mice on high-fat diet gained more weight than their control littermates, with reduced TG lipolysis observed in their adipocytes (35). Complementing the data...
from mouse studies, an epidemiological study comparing healthy controls with overweight and obese patients showed that ABCA1 expression was significantly decreased in the liver of overweight and obese subjects, whereas the levels of ABCG1 and SR-BI were comparable between the groups. Furthermore, the reduction in ABCA1 expression was associated with reduced cholesterol efflux from monocyte-derived macrophages (201).

To further explore the mechanistic involvement of HDL biogenesis in obesity, we employed apoA-I/H11002/H11002 mice. Again, we found that lack of classical apoA-I-containing HDL significantly affected plasma TG metabolism. These mice demonstrated enhanced intestinal absorption of dietary TG, accelerated clearance of postprandial TG, and a reduced rate of hepatic very low-density lipoprotein (VLDL) TG production (82). These mechanistic alterations in plasma TG metabolism correlated with increased diet-induced hepatic TG deposition and disturbed hepatic histology in apoA-I/H11002 mice compared with their wild-type counterparts, while they exhibited reduced glucose tolerance and insulin sensitivity (82). Interestingly, overexpression of apoA-I/ Milano, a gain-of-function mutant of apoA-I currently under pharmaceutical development, by adenovirus-mediated gene transfer in apoA-I/H11002 mice led to a significant reduction of hepatic lipid deposition and body weight gain. This observation further reinforced the notion that reduced plasma apoA-I levels are causative for increased body fat gain and hepatic lipid accumulation (82). In agreement with our published work, Liu et al. (123) showed that infection of C57BL/6 mice fed methionine and choline-deficient (MCD) diet for 1 wk with an adenovirus expressing wild-type human apoA-I led to a significant reduction of hepatic lipid deposition induced by MCD diet as well as hepatic cholesterol, TG, and fatty acid levels. Along the same lines, another study, by Wang et al. (196), showed that intravenous administration of varying doses of apoA-I in New Zealand White rabbits fed a high-fat diet for 20 wk resulted in an apoA-I dose-dependent reduction in hepatic steatosis, inflammation, hepatic lipid levels [cholesterol, TG, low-density lipoprotein-cholesterol (LDL-C)] and malondialdehyde (MDA) levels in the treated rabbits. In that study, the authors attributed the beneficial effect of apoA-I in NAFLD to its antioxidant properties, since important oxidative stress-related parameters were markedly inhibited in the apoA-I-treated animals. Specifically, apoA-I increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities while reducing inducible nitric oxide synthase (iNOS) activity, which can persistently and profusely release NO in serum and liver.

Contrasting the role of apoA-I in diet-induced obesity and hepatic lipid deposition, apoA-II, the second most abundant protein of HDL (18) appears rather to promote the development of obesity and NAFLD in apoA-II transgenic mice. Whole body fat content was found increased by almost 45% in the apoA-II transgenic mice fed a high-fat diet that could be fully accounted for by the percent increase observed in both retroperitoneal and epididymal fat pads (23). Interestingly, an epidemiological study among Egyptian adolescents suggested a possible relationship between the c.-492T>C polymorphism in human apoA-II gene with increased risk for obesity (204). Similarly, in another clinical study, in overweight African American and white women, it was found that the T265C apoA-II polymorphism correlated with increased visceral adipose tissue (VAT) mass in carriers of the C allele compared with noncarriers, but this observation was significant only in white women, where the mutant C allele in the apoA-II gene was more common (111). Apparently, however, the limited information in the literature on the potential role of apoA-II in HDL functionality did not make it possible in any of these epidemiological studies to associate polymorphisms with changes in the function of apoA-II that could provide mechanistic interpretation to their observations.

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plasma, was associated with altered plasma TG metabolism (81). Le<sup>cat</sup>−/− mice were also found prone to diet-induced obesity and hepatic lipid deposition, although those mice were resistant to the development of glucose intolerance. Adenovirus-mediated gene transfer of human le<sup>cat</sup> in le<sup>cat</sup>−/− mice fed a Western type diet for 12 wk resulted in a significant improvement of hepatic lipid deposition combined with body weight loss (81). Interestingly, LCAT and LDL receptor (LDLR) double deficient mice appear resistant to diet-induced obesity, but obviously this reflects a dominant role of LDLR in body weight gain and white adipose tissue mitochondrial metabolic activation recently seen in mice (29). In a more recent study, we also found that SR-BI, the final molecular partner of HDL metabolic pathway in plasma, regulates plasma apoE levels and postprandial TG metabolism in mice (83). Specifically, we found that feeding scarb1−/− mice a high-fat diet for 24 wk resulted in the gradual accumulation of apoE-containing HDL that ultimately results in insufficient LPL activity in circulation. In response, plasma TG levels increase along with the size of TG-rich lipoproteins, and subsequent LDLR-mediated clearance of apoE-containing TG-rich lipoproteins is impaired despite a compensatory increase in hepatic LDLR expression. Eventually, this leads to inhibition of dietary lipid shuttling to the liver and prevention of diet-induced obesity and NAFLD development (83). In agreement with these findings in mice, data from bariatric clinical trials reported that rapid weight loss following surgery was associated with increased SR-BI-mediated efflux of cholesteryl esters on HDL, leading to increased levels of larger cholesteryl ester-rich HDL2 particles (6, 210).

Recently, it was proposed that ectopically expressed mitochondrial ATP synthase (namely ecto-F1-ATPase) on cell membrane serves as an alternative cell-surface HDL receptor (185). Specifically, it was proposed that upon binding of apoA-I to ecto-F1-ATPase intracellular ATP is hydrolyzed to ADP, which is then released into circulation, and this hydrolysis fuels the activation of nucleotide receptor P2Y13 resulting in HDL holoparticle endocytosis (76, 130). The authors proposed this as an alternative to the SR-BI mechanism of active HDL holoparticle uptake. However, our alternative interpretation is that this mechanism may represent a way of activating cellular metabolism by replenishing intracellular ATP storage. It is possible that via this pathway HDL stimulates the consumption of intracellular ATP on cell membrane, thus promoting fat and glucose catabolism through activation of mitochondrial oxidative phosphorylation. The precise role of this new mechanism remains to be clarified.

**HDL in insulin resistance, glucose intolerance and type 2 diabetes mellitus.** Type 2 diabetes mellitus is a major global health problem, affecting over 300 million people worldwide (169). Type 2 diabetes mellitus develops as a result of reduced glucose tolerance, initially manifested as reduced peripheral insulin sensitivity and later on as impaired insulin production and secretion by pancreatic β-cells. Recently, a lot of attention has been placed on the role of HDL in the regulation of β-cell secretory function and peripheral tissue insulin sensitivity, and data from numerous studies strongly support that the pancreas, as well as skeletal muscle and adipose tissue, could benefit from improved HDL functionality (133).

It is generally believed that a correlation exists between HDL-C levels and the risk of developing type 2 diabetes mellitus (133, 138, 190). Some studies suggest that HDL contributes to euglycemia via both insulin-dependent and insulin-independent pathways (44, 191), while others indicate that insulin resistance may be causal for reduced plasma HDL-C levels through indirect mechanisms involving Toll-like receptors (TLRs), free fatty acids (FFA), various microRNAs (e.g. miR33) (192). Indeed, strong experimental data suggest that HDL has a direct effect on peripheral insulin sensitivity and glucose-stimulated pancreatic β-cell insulin secretion. Infusion of reconstituted HDL (rHDL) to patients with type 2 diabetes mellitus reduced plasma glucose levels through mechanisms involving glucose uptake in skeletal muscles (43, 74). In fact, glucose uptake was increased by human primary skeletal muscle cells when cultured with rHDL and apoA-I, in a dose-dependent manner (31). Besides, C57BL/6 mice that were injected with apoA-I increased their glucose disposal capacity and glucose-stimulated insulin secretion (166). It has been proposed that this may take place via 5′-adenosine monophosphate-activated protein kinase (AMPK) activation (32, 163), since apoA-I-deficient mice were found to have low levels of phosphorylated AMPK and demonstrated glucose intolerance (62). This agrees with previous observations that HDL increases intracellular calcium in skeletal muscle and stimulates Ca<sup>2+</sup>/calmodulin-stimulated kinase (CaMK) and AMPK activities (43). Additionally, recombinant human apoA-I and rHDL promote GLUT4 translocation to the cell membrane of skeletal muscle cells, thus providing a possible explanation as to why glucose uptake increases (32).

Interestingly, recent work from our laboratory revealed an important role of apoA-I in the modulation of the pharmacological actions of metformin, a common drug for the treatment of type 2 diabetes (80). Using experimental mouse models, we showed that deficiency in apoA-I ablates the pharmacological effects of metformin on plasma glucose homeostasis and hepatic lipid deposition, further reinforcing the important role of apoA-I in diabetes development and treatment. Of note, the effects of apoA-I deficiency on metformin action were AMPK independent, since in both C57BL/6 and apoA-I-deficient mice we observed similar levels of phosphorylated AMPK (80).

Interestingly, data suggest that apoA-I initiates a calcium-sensitive signaling cascade independently of ABCA1-mediated cholesterol efflux (43, 116). Hepatic ABCA1 deficiency in mice leads to low plasma HDL-C levels and impairment of glucose tolerance but normal insulin sensitivity, which may indicate that glucose intolerance results from decreased insulin secretion from β-cells (36). However, selective extraction of cholesterol from C2C12 myotubes had no effect on AMPK phosphorylation, further supporting that cholesterol efflux itself does not affect AMPK activation (62). In agreement with these data, a recent epidemiological study showed that genetic variations in ABCA1 and ABCG1 are not associated with peripheral insulin resistance (158). On the other hand, work in experimental mice and cultured pancreatic β-cells indicate an indirect involvement of ABCA1 in pancreatic β-cell function via reduction of intracellular free cholesterol levels and associated toxic effects to the cells (19, 36, 104, 105, 198). Further supporting this hypothesis, incubation of MIN6 cells with apoA-I and apoA-II, in either their lipid-free form or as a constituent of discoidal rHDL, or HDL from human plasma, also increase glucose-stimulated insulin secretion by promoting cholesterol efflux via ABCA1 or ABCG1, respectively (55). Another potential mechanism explaining the beneficial
effects of HDL on β-cell health may involve inhibition of cytokine-stimulated apoptosis. HDL appears to influence β-cell survival and function by protecting the cells from cholesterol-induced dysfunction, stress-induced apoptosis, and inflammation (103, 112, 152, 155).

Contrasting the view that HDL dysfunction is a factor contributing to diabetes via insulin-dependent and insulin-independent pathways, other data support that it is diabetes that renders HDL dysfunctional. Indeed, under conditions of hyperglycemia and hyperinsulinemia, carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element-binding protein-1c (SREBP1c) are activated in liver (50, 100, 160), and as a consequence, VLDL synthesis and secretion from the liver is increased, resulting in hypertriglyceridemia and enhanced activation of CETP (12, 50, 190). In turn, this promotes the enrichment of HDL particles with TGs while depleting them from cholesterol esters (50). This TG enrichment renders HDL as a preferred substrate for hepatic lipase (HL) that further reduces HDL core size. As a result, HDL-C levels decrease and the levels of minimally lipidated apoA-I increase. Thus, reduction of HDL core size leads to release of apoA-I from HDL and its subsequent excretion in the urine, explaining the lower plasma apoA-I levels seen in subjects with type 2 diabetes mellitus (12). There is also clinical evidence suggesting that not only low HDL-C levels but also HDL-C to apoA-I and apoA-II ratios may predict the risk of developing type 2 diabetes mellitus (1, 74). Additionally, nonenzymatic glycation of apoA-I that takes place in hyperglycemic conditions contributes to impaired cholesterol efflux and the antioxidant capacity of HDL as well as reduced activation of LCAT (137, 157). In particular, glycation end products are shown to impair cholesterol efflux by interacting not only with HDL particles but also with peripheral cells, where they decrease ABCA1 and SR-B1 expression (12, 45, 70, 138).

Collectively, the current literature indicates that high levels of functional HDL do improve glucose intolerance and insulin sensitivity. However, it appears that a self-feeding cycle exists where impaired HDL functionality reduces peripheral tissue insulin sensitivity and pancreatic β-cell function, which in turn causes further impairment in the HDL metabolic pathway. This impacts HDL levels, thus contributing to further glucose intolerance and progression of type 2 diabetes mellitus.

HDL in Gram-negative sepsis and inflammation. Bacterial sepsis is a leading cause of death in hospitalized patients. Mortality is largely due to the cytotoxic actions of lipopolysaccharide (LPS) present on bacterial outer membrane. Although LPS is not toxic when incorporated into the membrane, its release into the circulation following bacterial reproduction, lysis, or death triggers an inflammatory response. In particular, lipid A, a structural component of LPS (183), elicits a strong inflammatory response mediated by proinflammatory cytokine production, such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6), released mainly from monocytes/macrophages and neutrophils (183). LPS is identified mainly by TLR4 in cooperation with other proteins containing MD-2, CD14, and LPS-binding protein (LBP) (143). LBP catalyzes the transfer of LPS to CD14, thus promoting LPS-induced cell activation (77, 183). To avoid exaggerated responses to LPS, the host has developed several control mechanisms that contain inhibitory LPS-binding proteins and plasma lipoproteins (183). During severe sepsis, plasma HDL-C declines rapidly. Concomitantly, serum amyloid A (SAA) becomes the main protein component of HDL (45%) at the start of sepsis and is slowly replaced by apoA-I during recovery (184). Low HDL-C levels are inversely associated with the severity of septic disease and correlate with an exaggerated systemic inflammatory response (197). Also, in healthy subjects, low HDL levels are correlated with increased inflammatory response on endotoxin challenge compared with subjects with normal or high HDL levels (15), without differences in the HDL proteome (118). These observations suggest a positive role of HDL in the protection against sepsis.

Several mechanisms have been proposed for the HDL-mediated protection from sepsis. HDL, as well as other plasma lipoproteins (LDL, TG-rich lipoproteins), bind and neutralize Gram-negative bacterial LPS as well as Gram-positive bacterial lipoteichoic acid (LTA) (59, 134). apoA-I knockout mice, which lack HDL, exhibit reduced LPS neutralization in serum compared with control mice (60). On the other hand, overexpression of apoA-I moderately ameliorates their survival compared with controls, suggesting that HDL elevation may protect against septic death. In endotoxemic rats in which LPS has been infused after HDL administration, HDL prevents LPS-induced organ damage by lowering TNFα and NO production (114).

In addition to its role in LPS neutralization, HDL exerts its protective properties against sepsis also by promoting LPS clearance, in fact, almost all LPS in circulation appears bound to HDL (180, 181). HDL promotes SR-BI-mediated LPS uptake, since binding of the HDL-LPS complex to SR-BI intercedes LPS uptake (188). Administration of rHDL efficiently inhibits LPS-induced cytokine release from whole blood in vitro (143). Similarly, administration of rHDL to healthy volunteers by intravenous infusion before initiation of endotoxemia (141) significantly reduces clinical symptoms, and inflammatory cytokine (TNFα, IL-6, IL-8) production, and attenuates LPS-induced leukocyte activation, particularly due to the downregulation of the main LPS receptor, monocyte-bound CD14 (141).

In LPS-challenged macrophages, HDL selectively prevents the activation of type I interferon (IFN)-responsive genes (170), which have a critical role in the antiviral response of cells, although emerging evidence also implicates this response in host defense during bacterial infection. This inhibitory property of HDL does not require LPS binding to lipoproteins (170). HDL also attenuates LPS-induced neutrophil activation (135). One additional mechanism by which HDL expends its protective role against sepsis is by inducing an early inflammatory response to Gram-negative bacteria through suppression of the inhibitory activity of LBP (174), thus increasing the sensitivity of host response to LPS.

apoA-I plays a pivotal role in the anti-inflammatory functions of HDL and exhibits protective effects against sepsis. In fact, apoA-I directly inactivates bacterial endotoxin LPS by protein-protein interactions (47) through its COOH-terminal domain (65). apoA-I also prevents LPS-induced cytokine release from human monocytes (54) and decreases TNFα levels during LPS challenge in rats while increasing their survival (73). It has been proposed that apoA-I might inhibit LPS binding to macrophages, thus inhibiting the production of inflammatory cytokines that correlate with sepsis development.
and progression. In mice, apoA-I overexpression (which leads to increased serum levels of both apoA-I and HDL) attenuates LPS-induced acute injury in lung and kidney (120). LPS-induced proinflammatory cytokines, and CD14 expression in liver and lung decrease, suggesting a protective role of HDL against systemic inflammation and multiple organ damage (120). Similar to mouse data, human subjects with low plasma HDL levels (hypoprophilipoproteinemia) demonstrate an elevated prevalence of classical (phagocytic) CD14+/−/CD16− but not of intermediate CD14+/−/CD16+ monocytes, further linking HDL to LPS-mediated responses (156). It is interesting that septic HDL almost lacks apoC-I (8), another apolipoprotein that contains a consensus LPS-binding motif (13). Therefore septic HDL fails to inhibit the biological response to LPS and death in mice with Gram-negative-induced sepsis (13).

Similar to apoA-I, ABCA1 also appears to play an important role in Gram-negative-induced sepsis mainly by affecting the composition and integrity of lipid rafts on the cell membrane. Therefore, the anti-inflammatory activity of ABCA1 is secondary to its ability to regulate the cholesterol content of membrane lipid rafts. Lipid rafts contain an ordered membrane microdomain, which has plenty of free cholesterol, glycosphingolipid, and glycosylphosphatidylinositol-anchored proteins and are platforms for signal transduction (52, 148, 161). Mounting evidence proposes an association between the presence of lipid rafts in the plasma membrane and the proinflammatory stimulation of macrophages by TLR4 agonists such as lipopolysaccharide (LPS) (87, 140, 176). Indeed, HDL directly interacts with lipid rafts and effluxes cholesterol from these microdomains (131, 150). On the other hand, lipid efflux to purified apoA-I does not occur in lipid rafts (131), implying that apoA-I may remove cholesterol from other specialized domains of the plasma membrane. Interestingly, the expression of ABCA1, which is likely enriched in nonraft domains (66, 131), leads to a significant redistribution of free cholesterol and sphingomyelin from lipid rafts to nonrafts through its ATPase-related functions, and apoA-I preferentially correlates with nonraft membranes in ABCA1-expressing cells (110). These findings indicate that ABCA1 actively disrupts raft microdomains, altering free cholesterol redistribution from highly orderly lipid rafts to disordered nonraft regions. Free cholesterol in nonraft membrane compartments may be readily available to acceptors, thus promoting cholesterol-apoA-I interaction and lipid efflux.

Using macrophage-specific Abca1 knockout (Abca1−/−/−/−) mice, it was shown that Abca1-deficient versus wild-type macrophages had over 95% less ABCA1 protein, failed to efflux lipids to apoA-I, and showed a significant increase in free cholesterol and membrane lipid rafts without molecular signs of endoplasmic reticulum stress (207). Abca1-deficient macrophages displayed enhanced expression of proinflammatory cytokines in response to MyD88-dependent TLR (TLR2, TLR4, TLR7, and TLR9) stimulation but not MyD88-independent TLR3 stimulation (207, 208). In vivo LPS injection affirmed the proinflammatory response observed in Abca1−/−/−/− mice (207). Moreover, severe cholesterol depletion of macrophages with methyl-β-cyclodextrin normalized free cholesterol content between the two genotypes and their response to LPS, and cholesterol repletion of macrophages resulted in elevated cellular free cholesterol accumulation and enhanced cellular response to LPS, suggesting that lipid rafts play a key role in enhancing the proinflammatory response of Abca1-deficient macrophages (207). Lipid rafts from Abca1-deficient macrophages consisted of significantly more free cholesterol than wild-type macrophages and displayed a significantly increased TLR4, as well as TLR9 content (208), which partially explains the heightened inflammatory response of Abca1-deficient macrophages to TLR stimulation. These anti-inflammatory repercussions are not specific to ABCA1, since ABCG1, another member of the ABC transporter superfamily that stimulates cholesterol efflux to lipidated HDL, also reduces inflammatory responses in mice. In the absence of both ABCA1 and ABCG1, there is a far more pronounced proinflammatory response following TLR activation, due to increased TLR4 content of lipid rafts in macrophages (203). Thus, in normal macrophages, cholesterol efflux to apoA-I via ABCA1 or to HDL via ABCG1 efficiently protects cellular cholesterol homeostasis and ensures physiological content of plasma membrane lipid rafts.

Recently, we investigated the potential correlation between HDL particle quality and its anti-inflammatory functions in LPS-induced inflammation (145). As LCAT is a critical enzyme in HDL maturation, we investigated whether LCAT deficiency results in an altered LPS-induced inflammatory response. Our results showed that lack of functional LCAT markedly enhances and prolongs the LPS-induced inflammatory response in vivo via distinct mechanisms. Although serum and HDL from lcat−/− mice show reduced capacity to attenuate the LPS-induced TNFα response in vitro, deficiency of LCAT in vivo is associated with increased monocyte numbers, fewer proinflammatory monocytes, and dampened peritoneal macrophage responsiveness to LPS. Therefore, it appears that LCAT-mediated maturation of HDL plays a crucial role in neutralizing circulating LPS and in determining the number and phenotype of monocytes and macrophages in mice (145).

In conclusion, a critical review of the literature strongly favors the hypothesis that simply raising plasma HDL levels may not represent an effective therapeutic approach in the attenuation of sepsis and its complications. In the past, a number of HDL-based therapies have been proposed and have been tested mainly in vitro (61), in vivo in animal models (33, 205), or in humans (141, 159). Despite some promising early results, none of these approaches made it to the clinic, apparently due to the structural complexity of HDL that influences its antiinflammatory function.

HDL in bone physiology. Osteoporosis, a major public health problem worldwide, is characterized by low bone mass and microarchitectural deterioration of bone, resulting in bone fragility and fracture susceptibility (127, 162). Although it is well established that osteoporosis is a multifactorial disease, only recently has it become apparent that a strong link exists between bone and fat metabolism (28, 85, 202) and that perturbations in lipid metabolic pathways affect the balance between osteoblast and osteoclast function (28, 85, 121, 202, 206). In particular, differentiation of mesenchymal origin osteoblasts to adipoblasts has been correlated with an imbalance of bone formation and resorption, leading to bone loss. Several signaling pathways have been implicated in the adipogenic or osteogenic differentiation of mesenchymal stem cells, including peroxisome proliferator-activated receptor-γ (PPARγ), bone morphogenetic proteins (BMPs), and the Wnt signaling pathway (172).
More recent studies indicate that the relationship between fat and bone and between energy metabolism and bone is far more complex (22, 113). In particular, it was demonstrated in vitro and in vivo that apoE modulates bone mass and turnover (11, 39, 51). In addition, epidemiological studies showed that low levels of plasma HDL-C are strongly correlated with osteoporosis (3). Of note, SR-BI deficiency engenders dense bone (128, 129), which has been attributed to increased HDL-C delivery for cortisol production in the adrenal (128, 129).

In addition to osteoporosis, we recently demonstrated that apoA1−/− and lcat−/− mice develop osteoarthritis when fed a high-fat diet, as demonstrated by the presence of surface cartilage fibrillations, chondrocyte clustering and hypertrophy, loss of chondrocyte zonation, and proteoglycan depletion (177). Expression levels of type II collagen were significantly suppressed, while the levels of metalloproteinases (MMP)-2 and -9 as well as MMP-13 were significantly higher in the knee cartilage of lcat−/− and apoA1−/− obese mice compared with their lean littermates and C57BL/6 mice, respectively. This finding suggests that apoA-I and LCAT deficiency and, hence, the inability to produce HDL or mature HDL, predisposes to the development of osteoarthritis in mice following chronic insult by WTD (177). In addition, our data showed that bone marrow from lcat−/− and apoA1−/− mice fed WTD contained a significantly higher number of adipocytes than bone marrow from their lean counterparts. A plausible explanation is that adipocytes in bone marrow function in a fashion similar to adipocytes in white adipose tissue, thus producing and secreting adipokines (e.g., leptin, adiponectin, resistin) that may have an effect on articular cartilage associated with the development of osteoarthritis (113). Interestingly, we recently discovered that both apoA1−/− and lcat−/− mice, contain solely apoE-containing HDL particles and no apoA-I-containing HDL (145), which may explain the similar osteoarthritis pathophysiology between the two strains. Furthermore, this observation supports that apoE-containing HDL correlates with osteoarthritis development, reinforcing once again the notion that HDL quality, as determined by its apoprotein composition, is important in defining its relation with disease states. Collectively, these data raise the possibility that the reduced HDL seen in subjects with MetS is an additional parameter leading to osteoarthritis development, thus corroborating the hypothesis that osteoarthritis could be another constituent of MetS (186).

HDL in obstructive pulmonary disease. Obstructive pulmonary disease (COPD) has been widely recognized as a major cause of morbidity worldwide and is likely to be the third leading cause of death by the year 2020 (21). Chronic COPD (COPD) has been associated with several extrapulmonary systemic manifestations, including type 2 diabetes mellitus, osteoporosis, and MetS (125, 164). Although the coexistence of MetS and COPD is established, the pattern of dyslipidemia, a major feature of MetS, in COPD has not been well characterized. According to the CONSISTE study, dyslipidemia occurs in 48.3% of COPD patients compared with 31.7% in control subjects (34). In particular, elevated HDL-C levels have been observed in both early- and late-stage COPD patients (151, 175).

Interestingly, more recent studies revealed that the antioxidant potential of HDL in COPD patients, as measured by PON1 activity (2, 154) and HDL-C efflux capacity mainly via SR-BI (182) are reduced, indicating that HDL quality, rather than HDL-C quantity, is impaired in human COPD subjects. In support of this observation, it has been recently reported that serum levels of apoA-I and large HDL particles are also positively correlated with forced expiratory volume 1 (FEV1), whereas serum TGs, LDL-C, and apoB are associated with more severe airflow obstruction (9). Data in mice raise the possibility of a causative relationship between dysfunctional HDL and OPD. In particular, the anti-inflammatory action of apoA-I-containing HDL attenuates neutrophilic airway inflammation in experimental ovalbumin (OVA)-induced asthma by reducing the expression of granulocyte colony-stimulating factor (G-CSF) (30). Furthermore, ABCA1 expressed by vascular endothelial cells and alveolar macrophages contributes to the beneficial effect of apoA-I on the severity of neutrophilic airway inflammation in asthma (30).

HDL in brain physiology and disease. Growing evidence indicates that HDL has a substantial role in cognitive function, in aging, and in aging-related neurodegenerative disorders. This role relates mostly to its protein and lipid content and thus to its quality and functionality rather than to its quantity. Cholesterol is an essential structural and functional component of both peripheral and central nervous systems, with brain containing almost 25% of total body cholesterol (40), although it accounts for only 2% of total body mass (41). Seventy to 80% of brain cholesterol constitutes the structural component of myelin, where it serves as an insulator. The brain is isolated from peripheral blood circulation and consequently from peripheral organs by 1) the blood-brain barrier (BBB), formed by cerebrovascular endothelial cells (209), and 2) by the blood-cerebrospinal fluid barrier (BCSFB), composed of cuboidal epithelial cells of the choroid plexus (117). Free cholesterol cannot cross either the BBB or BCSFB (41); thus, all brain cholesterol originates from local de novo biosynthesis. However, it has been shown that some oxidized cholesterol metabolites, named oxysterols, can diffuse through the BBB to both directions and possibly fulfill novel, intriguing, and yet-unexplored roles in cholesterol signaling and homeostasis. (16, 17, 40). Brain cholesterol synthesis is regulated by mechanisms similar to those observed in peripheral organs and tissues, with hydroxymethyl-glutaryl CoA reductase (HMGCoA) activity being the rate-limiting step. Both in neurons and in glial cells, cholesterol synthesis is highly induced during embryogenesis and childhood when myellogenesis is underway. However, during adulthood, cholesterol biosynthesis in differentiated neurons progressively attenuates, and their ongoing cholesterol needs for maintenance and repair of damaged membranes are covered by glial cells (42).

Human CNS lipoproteins have been studied mainly in CSF, which is readily accessible by noninvasive methods. It is particularly important that apoB-containing lipoproteins, including LDL, VLDLs, and chylomicrons, do not cross the BBB or BCSFB (122). In addition, CSF lipoproteins have similar size and density to plasma HDL and thus are justifiably defined as “HDL-like particles” (92).

The particular lipid and protein components and characteristics of human CSF HDL have been studied extensively. Four distinct lipoprotein subpopulations have been identified: 1) the major subpopulation consisting of particles of 13–20 nm containing apoE, apoA-I, apoA-IV, apoD, and apoH, 2) a subpopulation of particles in the range of 13–18 nm, mainly composed of apoA-I and apoA-II but no apoE, 3) a small subpopulation...
of large particles (18–22 nm) containing apoE associated with apoA-IV, and apoD but no apoA-I, and 4) a fourth subpopulation containing small particles (10–12 nm) with low lipid content containing only apoA-IV, apoD, and apoH. All subpopulations contain similar amounts of apoJ. Notably, apoE and apoA-I are the major protein constituents of CSF HDL-like particles (92).

Human apoE in peripheral circulation cannot cross either BBB or BCSFB (122). Therefore, brain apoE is locally expressed by astrocytes and microglia (189) in three different isoforms (apoE2, apoE3, and apoE4), with a frequency of 7, 78, and 15%, respectively (167). Interestingly, it has been shown that adult glial cells also express apoD and apoJ (189). However, the brain does not produce apoA-I, and all brain apoA-I originates from the peripheral blood circulation (38, 41, 92). From a mechanistic point of view, Balazs et al. (2004) showed that small apoA-I-containing HDL particles can transcytose across an in vitro BBB and that caveoleae-located SR-BI facilitates this selective process (7). In addition, it has been reported that apoA-I is expressed by brain capillary endothelial cells that constitute the BBB (142). However, it has been found that plasma apoA-I-HDL and CSF apoA-I-HDL levels show a close correlation (49), suggesting that plasma apoA-I-HDL levels could modulate brain apoA-I-HDL levels. Moreover, it has been shown that both apoA-I (75) and apoE-containing lipoproteins (195) of human CSF are capable of promoting cholesterol efflux from rat astrocytes, with apoA-I-containing HDL being a far better inducer of cholesterol efflux than apoE-containing HDL (38).

Although the mechanism underlying the synthesis of nascent lipoproteins in the brain remains vague, experimental evidence has shown that cultured astrocytes can synthesize discoaloid apoE- and apoJ-containing lipoproteins (109). Moreover, plasma-derived apoA-I-containing discoaloid HDL particles may enter the CNS via SR-BI-mediated transcytosis in the BBB (7). Furthermore, the brain also expresses lipoprotein-related enzymes [e.g., LCAT (67)], ABC transporters [e.g., ABCA1 (69, 95), ABCG1 (25), ABCG4 (25), and ABCA8 (91)], and lipid transfer proteins [e.g., PLTP (26) and CETP (5)], which have been hypothesized to promote HDL particle remodeling in a fashion analogous to that in peripheral circulation (72, 189). However, the precise role of each one of these molecular partners of HDL metabolic pathway has not been studied in detail.

It has been proposed that in brain parenchyma ABCA1 transporters expressed in neurons and astrocytes promote the efflux of phospholipids and unesterified cholesterol to glia-derived apoE, giving rise to nascent apoE-containing discoaloid HDL. Both glia-derived apoE-containing discoaloid HDL and plasma-derived apoA-I-containing discoaloid HDL can further acquire phospholipids and unesterified cholesterol via ABCA1 and ABCG1 in glia and via ABCA1 and ABCG4 in neurons. Maturation of discoaloid HDL particles into spherical ones involves most probably the action of LCAT, and subsequent remodeling of PLTP and CETP, in a way similar to that in peripheral circulation. Yet, the different apolipoprotein content of brain HDL-like particles sets the basis for structural differences between these particles. Specifically, apoA-I-containing particles are smaller and phospholipid rich, whereas apoE-containing particles are bigger and cholesterol rich (75, 92, 149). Mature brain HDL-like particles are then taken up by neurons and astrocytes through binding of apoproteins to specific receptors. Indeed, the brain expresses several apoE receptors [e.g., LDLR (24), LRP1 (146), VLDLR (178), and apoER2 (90)], as well as the apoA-I receptor SR-BI (4).

Recent data indicate that brain HDL may play a role in dementia, which in medical terms is a loss of mental ability that interferes with normal daily activities and manifests a group of symptoms caused by gradual death of brain cells. The loss of cognitive function that occurs with dementia leads to impairments in memory, reasoning, planning, and behavior. Alzheimer’s disease (AD) accounts for 60–80% of dementia cases. Vascular dementia, which occurs after a stroke, is the second most common dementia type (http://www.alz.org). Additionally to AD, other progressive neurodegenerative disorders are highly correlated, albeit at a different presentation incidence, with dementia development, including Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), multiple system atrophy (MSA), and multiple sclerosis (MS). Of note, all the above-mentioned degenerative disorders have also been related to low HDLc and/or apoA-I levels in blood circulation and/or CSF (72, 189).

Given that apoA-I is the only known protein partner of peripheral lipoprotein metabolism not expressed in brain and that can cross the BBB and BCSFB in the form of discoaloid HDL, it may serve as the molecular link between elevated plasma HDL levels and improved brain cognitive function. However, the exact mechanism underlying this association remains elusive, although, as described below, different brain HDL functions correlate closely with different disease states.

It is well established that ABCA1 is expressed in all brain cell types, where it regulates cholesterol efflux to lipid-free apolipoproteins, thus mediating cholesterol transport from glial cells (mainly astrocytes) to neurons (95). Recent experimental evidence revealed a significant decrease in neurite length and number of neurite segments in the CA1 region of the hippocampus of abca1−/− mice, suggesting the importance of Abca1 in neurite degeneration in the brain (93). Moreover, the lack of functional ABCA1 affects apoE lipidation and stability, leading to a significant decrease of apoE levels in the brains of abca1−/− mice. Mice with a targeted disruption of brain abca1 had a significant reduction of apoE in the CNS and a very low level of apoE-HDL in the CSF (79). The particular effect of ABCA1 appears to be apoE selective, since the levels of apoJ are independent of ABCA1 expression (68). In addition, it has been reported that human CSF-derived apoA-I also promotes cholesterol efflux from astrocytes through ABCA1 and forms discoaloid, phospholipid-rich HDL-like particles (75).

It has been proposed that brain HDL-like particles may influence AD neurodegeneration as carriers of cholesterol (46). In particular, one of the principal mechanisms proposed to affect APP processing to amyloidogenic or nonamyloidogenic cleavage is the modulation of membrane fluidity by apoA-I-mediated cholesterol efflux (38, 199). However, once β-amyloid (Aβ) is produced, it binds to HDL and maintains its solubility in CSF and plasma. This HDL-Aβ interaction could prevent the deposition of Aβ into the brain and may serve as a marker for neurodegenerative disease (96, 101). In support of this hypothesis, recent data in mice have shown that lack of apoA-I exacerbates the cerebrovascular deposition of Aβ (115), whereas apoA-I overexpression ameliorates it (119). Interestingly, apoE has been proved not to have a significant
interaction with Aβ; instead, it has been shown to compete with Aβ for the same clearance pathways (71, 78, 124, 187). Taken together, all these data strongly indicate that HDL quality in terms of its apolipoprotein content plays a pivotal role in modulating Aβ clearance and consequently AD pathogenesis.

A key step in HDL metabolism is particle conversion from discoidal to spherical through esterification of free cholesterol by LCAT (67). apoE is presumed to be one of the most potent LCAT activators in the CNS, as it is sufficient to stimulate esterification of endogenous cholesterol in glial-conditioned media (48). apoA-I, however, is also capable of promoting the esterification of free cholesterol by glial-derived LCAT (67). Given the particular role of apoE in the pathological progression of Alzheimer’s disease, including facilitating Aβ clearance from the brain, a function that requires its lipidation by ABCA1 (68, 94, 193, 194), Stukas et al. studied how LCAT deficiency influences CNS lipoprotein metabolism and amyloid pathology. Although, deletion of LCAT from APP/PS1 mice, an experimental model of AD, resulted in a pronounced decrease of apoA-I in plasma CSF, and brain apoE levels were unaffected. Furthermore, LCAT deficiency did not increase Aβ or amyloid in APP/PS1 LCAT−/− mice. Taken together, these results show that efficient Aβ clearance via apoE-containing discoidal HDL does not require LCAT (168). Although LCAT deficiency does not affect Aβ metabolism in this particular AD mouse model, it may influence other important brain functions. Indeed, traumatic brain injury in humans, for example, leads to a significant decrease in CSF apoE levels but not in CSF apoA-I levels (86), whereas loss of apoE impairs recovery after traumatic brain injury in mice (136).

The antioxidant action of HDL in periphery is well established (84, 179), and we recently showed that this particular HDL function is strongly related to HDL protein content and consequently to its quality (210). Interestingly, Paterno et al. (2004) showed that the beneficial effect of reconstituted human apoA-I-containing HDL on neuronal damage after stroke occurs through antioxidative mechanism(s), highlighting the importance of the antioxidative action of apoA-I-containing HDL in brain physiology and metabolism (144).

Since inflammation has been proposed to influence the development of AD (200), it has been suggested that the anti-inflammatory action of apoA-I-containing HDL may also play a significant role in protection against neurodegeneration (72, 189). Although studies are still limited, it has been shown that overexpression of human apoA-I attenuates neuroinflammation in the mouse AD model and improves cognitive function (119), whereas D-4F, an apoA-I mimetic peptide, proved to inhibit inflammation in brain and also improves memory deficits in mice (20, 63). Moreover, using HDL and apoA-I isolated from healthy subjects and AD patients, Khalil et al. (2012) demonstrated that the functionality of HDL from AD patients was impaired as a result of increased oxidative stress and inflammation related to AD (89). These findings strongly suggest that apoA-I-containing HDL exerts an important, yet understudied, anti-inflammatory role in neuroprotection.

**Future perspectives.** Accumulating new evidence in the literature strongly supports a causative role of dysfunctional HDL in a number of metabolic and brain disorders (Figs. 1 and 2). In particular, alterations in the HDL metabolic pathway as well as in the HDL proteome appear to influence to a great extent its properties and functions. However, most of the studies establishing such a structure-function relationship in HDL have been performed either in experimental animals or in cell cultures, with only limited, if any, such information validated in humans. Another significant missing part of the HDL puzzle is the correlation between HDL lipid content and particle functionality that to this day remains vague, since lipidomics studies have provided inconclusive information thus far (97–99). The proper understanding of the structure-function relationship of HDL will set off a revolution not only for the pathophysiology of diseases influenced by HDL but also for the development of effective pharmaceuticals that will target HDL functionality rather than HDL-C quantity. Although to this date there are no data from genome-wide association studies correlating genetic polymorphisms with HDL functionality, it is our expectation that this may change in the future. As focus shifts from HDL-C levels to HDL function and its relation to disease, such markers will be invaluable for predicting the risk for developing HDL-related disorders.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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