Novel Biological Functions of High-Density Lipoprotein Cholesterol

Chieko Mineo, Philip W. Shaul

Abstract: In addition to its role in reverse cholesterol transport, high-density lipoprotein (HDL) cholesterol has direct action on numerous cell types that influence cardiovascular and metabolic health. Cellular responses to HDL entail its capacity to invoke cholesterol efflux that causes signal initiation via scavenger receptor class B, type I, and plasma membrane receptor activation by HDL cargo molecules. In endothelial cells and their progenitors, HDL attenuates apoptosis and stimulates proliferation and migration. HDL also has diverse anti-inflammatory actions in both endothelial cells and leukocytes. In vascular smooth muscles, HDL tempers proinflammatory, promigratory, and degradative processes, and through actions on endothelium and platelets HDL is antithrombotic. There are additional actions of HDL of potential cardiovascular consequence that are indirect, including the capacities to promote pancreatic β-cell insulin secretion, to protect pancreatic β cells from apoptosis, and to enhance glucose uptake by skeletal muscle myocytes. Furthermore, HDL decreases white adipose tissue mass, increases energy expenditure, and promotes the production of adipose-derived cytokine adiponectin that has its own vascular-protective properties. Many of these numerous actions of HDL have been observed not only in cell culture and animal models but also in human studies, and assessments of these functions are now being applied to patient populations to better-fulfill which actions of HDL may contribute to its cardioprotective potential and how they can be quantified and targeted. Further work on the many mechanisms of HDL action promises to reveal new prophylactic and therapeutic strategies to optimize both cardiovascular and metabolic health. (Circ Res. 2012;111:1079-1090.)

Key Words: endothelium ■ high-density lipoprotein cholesterol ■ nitric oxide synthase ■ scavenger receptor class B, type I

Studies of the relationship between circulating concentrations of high-density lipoprotein (HDL) cholesterol and atherosclerosis and cardiovascular events have suggested that disease risk is inversely related to HDL level,

and that the association is independent of low-density lipoprotein (LDL) cholesterol. Even in patients treated aggressively with statins to decrease circulating LDL levels to <70 mg/dL, HDL may continue to be inversely related to the risk of major cardiovascular events. However, recent genetic analyses of a relatively common single nucleotide polymorphism in the endothelial lipase gene and other single nucleotide polymorphisms associated with HDL cholesterol suggest that genetic mechanisms that increase plasma HDL do not decrease the risk of myocardial infarction. In contrast, a previous study of single nucleotide polymorphisms in the cholesteryl ester transfer protein that impact HDL levels indicated that single nucleotide polymorphism associated with an increase in HDL corresponded with a lower risk of future myocardial infarction. Certain previous clinical trials with agents that increase HDL showed that elevations in the lipoprotein can decrease the incidence of cardiovascular events. However, in a recent study, the addition of extended-release niacin to simvastatin treatment to increase low levels of HDL did not impact residual cardiovascular disease risk. In addition, although potential off-target activities of the intervention likely cloud the interpretation of its findings, the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial testing the impact of the cholesteryl ester transfer protein inhibitor torcetrapib on clinical outcome showed an increase in cardiovascular events and total mortality, despite elevations in HDL cholesterol. Considering these cumulative observations and the outcomes of numerous other investigations that have interrogated the level of circulating HDL cholesterol as a disease risk marker, genetic mechanisms that influence HDL level and disease incidence, or the impact of an intervention to increase HDL on clinical outcome, it remains uncertain whether HDL cholesterol directly impacts atherosclerosis and the risk of cardiovascular events. From a mechanistic perspective, HDL classically functions in reverse cholesterol transport (RCT), removing cholesterol from peripheral tissues and cells such as macrophages, and delivering it to the...
liver and steroidogenic organs by binding of the major HDL apolipoprotein, apolipoprotein (apo) A-I, to the high-affinity HDL receptor scavenger receptor, class B, type I (SR-BI). In mouse models of atherosclerosis, both apoA-I and SR-BI provide atheroprotection, and the provision of apoA-I or HDL also attenuates neointima formation after artery injury in the context of experimental hypercholesterolemia. The potential protective nature of HDL has been primarily attributed to its role in RCT. However, the mechanisms by which HDL may impact cardiovascular health and disease remain complex and not fully understood, and it has become quite apparent that alternative mechanisms of action of HDL must be considered.

Our understanding of how HDL potentially modifies cardiovascular disease risk or outcome has expanded well beyond its participation in RCT with the discovery that HDL has direct actions on endothelial cells and other cell types that influence vascular health and disease. This review highlights advances in this aspect of HDL biology by summarizing findings from experimentation in cell culture and animal models and from observational and interventional studies in humans. Direct actions of HDL on cell types of relevance to vascular health and disease are reviewed first, followed by functions of HDL that may indirectly influence events in the vascular wall. In particular, the regulation of endothelial cell apoptosis, proliferation, and migration by HDL and HDL anti-inflammatory actions in both endothelial cells and leukocytes are discussed. HDL modulation of vascular smooth muscle (VSM) and platelet function also are considered. Because nitric oxide (NO) derived from endothelial NO synthase (eNOS) has both autocrine and paracrine influences on numerous vascular cell types, the capacity of HDL to modify eNOS subcellular localization, enzymatic activity, and expression are highlighted. More recently, appreciated actions of HDL of relevance to glucose homeostasis and adiposity also are discussed. Furthermore, the basis for intracellular signaling initiated by HDL is highlighted, and current knowledge gaps in this realm of HDL biology are considered.

Regulation of Endothelial Cell Apoptosis, Proliferation, and Migration

Endothelial Cell Apoptosis

Endothelial cell apoptosis contributes to the pathogenesis of atherosclerosis and other vascular disorders. An intact endothelial cell monolayer plays a critical role in normal homeostasis in the vascular wall, and apoptosis of endothelial cells can occur on exposure to circulating factors and inflammatory cells, leading to disruption of endothelial monolayer integrity. Multiple proatherogenic factors promote apoptosis in endothelium, and these include oxidized LDL (oxLDL) and tumor necrosis factor-α. OxLDL causes a delayed but sustained increase in intracellular calcium in endothelial cells, which results in cell death, and this effect is reversed by HDL and mediated by prevention of the calcium increase. The protection afforded by native HDL is mimicked by purified apoA-I, and it requires HDL binding to the cells and new protein synthesis. Tumor necrosis factor-α–induced endothelial cell apoptosis is also inhibited by HDL, and this is associated with attenuated induction of CPP32-like protease (caspase 3), which is a component of all primary apoptotic pathways. Growth factor deprivation–related apoptosis of endothelial cells is also suppressed by HDL. This is because of blunting of the mitochondrial pathway of apoptosis, with HDL attenuating the dissipation of mitochondrial potential, oxygen-derived free radical generation, cytochrome c release to the cytoplasm, and activation of caspase 3 and caspase 9. HDL also activates Akt and causes phosphorylation of the Akt target Bcl-2-associated death promoter, which favors Bcl-2-associated death promoter dissociation from B-cell lymphoma-extra large that is then free to inhibit mitochondria-mediated apoptosis. The HDL-associated lysophospholipids sphingosylphosphorylcholine and lysosulfatide protect endothelial cells from growth factor deprivation–related apoptosis via mechanisms paralleling those of native HDL. In addition, the lysophospholipid sphingosine-1-phosphate (S1P) enhances endothelial cell survival, with effects comparable with those of native HDL, and these responses are inhibited by knockdown of the S1P receptor endothelial differentiation gene-1/S1P1, by pertussis toxin and by phosphoinositide 3 (PI3) kinase and Erk pathway antagonists, suggesting that signaling by lysophospholipid components of HDL may be important for the inhibition of apoptosis. Although the majority of evidence for antiapoptotic action of HDL on endothelium comes from cell culture work, in studies of the apoA-I mimetic D-4F in a rat model of diabetes mellitus, the mimetic improved vascular reactivity and decreased endothelial cell fragmentation and sloughing. This finding suggests that the antiapoptotic actions of HDL may be operative in vivo, but additional in vivo evidence is needed.

The potential role of SR-BI in the modulation of endothelial cell apoptosis has been addressed in one report that involved heterologous expression experiments and studies in SR-BI−/− versus SR-BI+/− fibroblasts and endothelial cells. This work found that a putative redox motif CXXS residing at amino acids 323 to 326 of SR-BI may induce a novel ligand-independent apoptotic pathway mediated by caspase 8. The proapoptotic effects of SR-BI were reversed by HDL and eNOS, and the authors proposed that under normal conditions the antiapoptotic actions of NO prevail; however, with low levels

Non-standard Abbreviations and Acronyms

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<tr>
<th>Abbreviation</th>
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<tr>
<td>ABCA1</td>
<td>ATP-binding cassette transporter 1</td>
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<tr>
<td>apoA-I</td>
<td>apolipoprotein A-I</td>
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<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<td>EPC</td>
<td>endothelial progenitor cell</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>LDL</td>
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<td>oxLDL</td>
<td>oxidized low-density lipoprotein</td>
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<td>MAP</td>
<td>mitogen-activated protein</td>
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<td>MDA</td>
<td>malondialdehyde</td>
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<td>PON 1</td>
<td>paraoxonase 1</td>
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<tr>
<td>PI3</td>
<td>phosphoinositide 3</td>
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<tr>
<td>RCT</td>
<td>reverse cholesterol transport</td>
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<td>rHDL</td>
<td>reconstituted high-density lipoprotein</td>
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<tr>
<td>S1P</td>
<td>sphingosine-1-phosphate</td>
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<tr>
<td>SR-BI</td>
<td>scavenger receptor, class B, type I</td>
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<td>VSM</td>
<td>vascular smooth muscle</td>
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of HDL, SR-BI may promote endothelial cell death. This work is yet to be replicated and, as such, whether SR-BI actually has the potential to invoke apoptosis or affords protection from apoptosis in endothelial cells is yet to be clarified.

Endothelial Cell Proliferation and Migration

The processes of endothelial cell proliferation and migration are crucial to both neovascularization and a successful response to vascular injury. An intact endothelial cell monolayer modulates local hemostasis and thrombolysis and provides a nonpermeable barrier protecting VSM cells from circulating growth-promoting factors. Disruptions of endothelial cell monolayer integrity, either by gross denudation related to a vascular intervention or by gap formation between cells because of disturbed shear stress, place the arterial wall at greater risk for vascular disease. Whereas repeated endothelial removal worsens the severity of vascular lesions, enhanced reendothelialization blunts lesion formation.

Providing another potential mechanism whereby HDL may afford cardiovascular protection, in the 1980s it was demonstrated that the lipoprotein promotes endothelial cell proliferation and that the proliferative response is calcium-dependent. In 1994, it was further reported that HDL stimulates endothelial cell migration, independent of cell proliferation. In those early studies, the responses to HDL and basic fibroblast growth factor were additive, and the former response was not sensitive to pertussis toxin or inhibition of phospholipase A2, whereas the latter was sensitive, indicating independent signaling mechanisms. In contrast, in later studies HDL activation of endothelial cell migration was found to be prevented by pertussis toxin, mediated by the G-protein–coupled S1P receptors endothelial differentiation gene-1/S1P1 and endothelial differentiation gene-3/S1P3 and derived from the S1P-rich and not the remaining fractions of HDL. Dependence on PI3 kinase, p38 mitogen-activated protein (MAP) kinase, and Rho kinase also was observed. Capillary tube formation stimulated by HDL in vitro is pertussis toxin–sensitive but does not occur through p38 MAP kinase and alternatively requires p42/44 MAP kinase activity residing downstream of Ras. It also has been observed that HDL stimulates endothelial cell migration in vitro in an NO-independent manner via SR-BI–mediated activation of Rac GTPase. This process does not require HDL cargo molecules and is dependent on the activation of Src kinases, PI3 kinase, and p44/42 MAP kinases. Rapid initial stimulation of lamellipodia formation by HDL via SR-BI, Src kinases, and Rac also occurs in cultured endothelial cells. Thus, numerous studies have consistently demonstrated that HDL stimulates endothelial cell proliferation and migration. In contrast, there is inconsistency in the signaling mechanisms that have been implicated in these processes. Recognizing that few experiments have been performed in vivo, these inconsistencies may relate to the diversity of endothelial cell types that have been studied in culture and also variance in the experimental conditions used.

Complementing its actions on differentiated endothelial cells, HDL modifies endothelial progenitor cell (EPC) differentiation and function. Although initially believed that circulating EPCs contribute to endothelial repair by differentiating into endothelial cells, it is more likely that EPCs facilitate vascular repair and neovascularization by secreting paracrine factors. Reconstituted HDL (rHDL) induces Akt phosphorylation in human peripheral mononuclear cells and promotes their differentiation into EPC in a PI3 kinase–dependent manner. Mirroring the mechanisms by which HDL activates endothelial cell migration, the activation of EPC proliferation, migration, and tube formation by HDL in culture is dependent on SR-BI, PI3 kinase/Akt, MAP kinase, and eNOS. In a hindlimb ischemia model of neovascularization in mice, rHDL infusion causes an increase in capillary density and an augmentation in blood flow recovery that is eNOS-dependent. In addition, rHDL causes an increase in the number of EPCs detected in the aortic endothelium of apoE−/− mice. Paralleling these findings, carotid artery reendothelialization after perivascular electric injury is diminished in apoA-I−/− mice, reconstitution of apoA-I expression in apoA-I−/− rescues normal reendothelialization, and reendothelialization is impaired in SR-BI−/− mice. Furthermore, a role for SR-BI in EPC modulation by apoA-I/HDL has been demonstrated; however, adenosine-driven hepatic expression of human apoA-I causes an increase in circulating EPCs in wild-type mice transplanted with SR-BI−/− bone marrow and the EPC response is absent in mice transplanted with SR-BI−/− bone marrow. In the former group, but not in the latter, adenosine-driven human apoA-I expression also stimulates EPC incorporation and endothelial regeneration in paratopically transplanted carotid arteries. Comparable findings regarding human apoA-I transfer and endothelial regeneration have been obtained in a mouse model of transplant atherosclerosis in which apoA-I also blunts neointima formation.

Thus, HDL causes enhanced EPC differentiation and function and also stimulates endothelial cell migration via SR-BI–initiated signaling. There is evidence that these mechanisms promote endothelial monolayer integrity under a variety of experimental conditions in vivo.

Anti-Inflammatory Actions in Endothelium and Leukocytes

HDL has anti-inflammatory actions in both endothelial cells and leukocytes. In endothelial cells, the lipoprotein inhibits adhesion molecule expression, and this is mediated by SR-BI, the SR-BI adaptor protein PDZK1, PI3 kinase, eNOS, and, in some studies, also by S1P receptors, implicating participation by S1P. Studies in rabbits have demonstrated that vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 expression in the aortic endothelium is blunted by HDL in vivo and that the glycation of apoA-I, which occurs in hyperglycemic individuals with diabetes mellitus, impairs this anti-inflammatory property of apoA-I/HDL. HDL also directly attenuates the activation of monocytes/macrophages and neutrophils. In monocytes, both native HDL and apoA-I blunt phorbol myristate acetate induction of the integrin CD11b, which promotes adhesion and migration, and thereby decrease phorbol myristate acetate–related enhancement of monocyte-endothelial cell interaction. HDL also inhibits the binding of T-cell microparticles to monocytes and thereby blunts proinflammatory cytokine production. ApoA-I also attenuates dendritic cell differentiation from monocytes. In macrophages, apoA-I actions via ATP-binding cassette
vascular disorders. In neutrophils, native HDL and apoA-I decrease the surface expression of CD11b, and they do so via SR-BI and ABCA1, respectively. In parallel, HDL and apoA-I decrease neutrophil spreading and migration and neutrophil-platelet interaction. In an in vivo mouse model of inflammation, apoA-I attenuates endothelial cell–leukocyte adhesion assessed by intravital microscopy, and the infusion of rHDL into human subjects with peripheral vascular disease attenuates neutrophil activation. Interestingly, whereas mice receiving bone marrow transplantation with ABCA1-deficient and ABCG1-deficient marrow display leukocytosis, the leukocytosis is markedly suppressed in apoA-I transgenic mice, indicating that HDL additionally inhibits the proliferation of hematopoietic stem cells and multipotential progenitor cells. Therefore, there is both cell culture and in vivo evidence that through diverse actions in several cell types HDL has anti-inflammatory properties. The net effect of these processes on cardiovascular conditions that entail inflammation, however, is poorly understood. How HDL influences infectious processes, including hepatitis C virus infection, is not covered in this review but is summarized elsewhere.

Regulation of VSM Cells
Studies in cell culture indicate that HDL has numerous direct actions on VSM cells. One action is to enhance VSM cell prostacyclin production, and this results from the upregulation of cyclooxygenase type 2 expression. HDL also inhibits VSM cell migration via S1P-mediated processes. Through its S1P cargo, HDL additionally blunts the expression of monocyte chemoattractant protein-1 in VSM cells, and this is mediated by SR-BI, the S1P3 receptor for S1P, and an attenuation of reactive oxygen species production that regulates monocyte chemoattractant protein-1 production. The influence of HDL on reactive oxygen species production in VSM is through prevention of the activation of Rac1 and resulting inhibition of nicotinamide adenine dinucleotide phosphate-oxidase. Studies of VSM cells have further revealed that HDL has actions on the extracellular matrix. Mass spectrometry and immunoblotting have demonstrated the presence of α1-antitrypsin in HDL, and HDL has antielastase activity that blunts extracellular matrix degradation, cell detachment, and apoptosis induced by elastase in human VSM cells. The relative abundance of α1-antitrypsin in HDL is decreased in patients with abdominal aortic aneurysm compared with controls, suggesting that HDL from the aneurysm patients is less capable of inhibiting elastase. Thus, HDL tempers proinflammatory, promigratory, and degradative processes in VSM cells and, along with actions on endothelial cells and leukocytes, these mechanisms may impact atherosclerosis and other vascular disorders.

Regulation of Platelets
Platelet aggregation is inversely correlated with HDL levels in humans, suggesting that HDL has antiplatelet actions. The inhibition of platelet aggregation has been observed with both the infusion of rats with apoA-I Milano, which is a naturally occurring mutant form of apoA-I discovered in a village in Italy, and the administration of rHDL to humans, further supporting the concept that HDL inhibits platelet activation in vivo. Mechanistically, whereas HDL may reduce platelet activation directly, HDL also may act indirectly on platelet aggregation via one or more of its effects on endothelial cells. In particular, HDL potentially influences platelet function by downregulating the release of platelet-activating factor from endothelial cells or by activating eNOS. In the latter case, the increase in bioavailable NO inhibits the release of Weibel-Palade bodies and thus interferes with the activation of the endothelium required for platelet–endothelial cell interactions and thrombosis. HDL also downregulates endothelial cell thromboxane A2 synthesis and upregulates endothelial cell prostacyclin production, which can decrease platelet aggregation. Furthermore, endothelial cell expression of tissue factor, which plays a key role in the initiation of coagulation, is attenuated by HDL. Thus, the potential antithrombotic actions of HDL are multiple.

Modulation of eNOS
The autocrine and paracrine actions of endothelium-derived NO influence vascular cells in numerous ways. Because HDL activation of eNOS and resulting eNOS-dependent processes are becoming potentially important means of assessing the function of the lipoprotein in the clinical setting, the modulation of eNOS by HDL is reviewed in considerable detail.

Impact on eNOS Localization
In endothelial cells, eNOS is targeted to plasma membrane caveolae, which are a subset of lipid rafts, by both N-terminal myristoylation and palmitoylation. Caveolae are enriched in cholesterol, glycosphingolipids, sphingomyelin, and lipid-anchored membrane proteins; they compartmentalize a variety of signal transduction molecules and their function is highly dependent on their cholesterol content. Cell culture experiments have revealed that oxLDL causes depletion of caveolae cholesterol in endothelial cells via the scavenger receptor CD36, leading to ineffective eNOS targeting to caveolae and an attenuated capacity to activate the enzyme. Comparable findings have been obtained in vivo in studies of eNOS localization and function in apoE−/− versus apoE−/− CD36−/− mice. In the cell culture paradigm, whereas oxLDL alone disrupts normal eNOS subcellular targeting and function, the addition of HDL to the medium containing oxLDL prevents eNOS displacement from caveolae and restores acetylcholine-induced stimulation of the enzyme. The normalization afforded by HDL is because of prevention of the decline in caveolae sterol content caused by oxLDL, and this is mediated by an SR-BI–dependent provision of cholesterol esters from HDL to the caveolae membrane. In additional studies, the apoA-I mimetics L-4F and D-4F protected endothelial cell function by preventing LDL from uncoupling eNOS activity to favor superoxide anion production over NO production. These processes involving the diminution of the adverse impacts of LDL on the endothelium may explain at least a portion of the antiatherogenic potential of HDL.
Stimulation of eNOS Enzymatic Activity

In addition to normalizing the subcellular localization of the enzyme when the lipid environment within caveolae/ lipid rafts is overtly perturbed, HDL is a potent agonist of eNOS. Both heterologous expression experiments and studies of endothelial- and NO-dependent relaxation of aortic rings from wild-type versus knockout mice revealed that SR-BI mediates the activation of eNOS by HDL. The functional coupling of SR-BI to eNOS is demonstrable in isolated endothelial cell caveolae, indicating that all the molecular machinery required for HDL-induced eNOS stimulation is associated with caveolae.71

In both human and animals studies, apoA-I is the apolipoprotein principally responsible for the atheroprotective features of HDL.88 In cultured endothelial cells, potential apoA-I–eNOS interaction and perinuclear colocalization have been reported.89 However, eNOS enzyme activation has not been observed with lipid-free apoA-I, and corroborating evidence of this interaction is lacking. In isolated endothelial cell plasma membranes, anti-apoA-I antibody blocks eNOS activation by HDL, whereas anti-apoA-II antibody causes enhanced eNOS stimulation by HDL. Thus, apoA-I is necessary but not sufficient for eNOS stimulation, and apoA-II may negatively influence eNOS activation by yet-to-be-determined mechanisms.71 The impact of apoA-II on HDL modulation of eNOS or resulting processes has not yet been investigated in vivo.

eNOS activity is regulated by complex signal transduction pathways, which include the activation of kinases that alter the phosphorylation state of the enzyme. Akt kinase activates eNOS by directly phosphorylating the enzyme at Ser1179 (in bovine eNOS, eNOS-Ser1177 in humans, and eNOS-Ser1176 in mice). Akt itself is phosphorylated and activated by PI3 kinase, which in turn is activated by a tyrosine kinase, and both receptor tyrosine kinase and nonreceptor tyrosine kinase participate in eNOS activation by various agonists. In contrast to eNOS-Ser1179, the phosphorylation of eNOS-Thr497 (in bovine eNOS, eNOS-Thr495 in humans) attenuates enzyme activity under certain conditions. eNOS also is modulated by MAP kinases and, unlike Akt, the effect of MAP kinases on eNOS activity can be either positive or negative. In addition, there is a critical role for calcium-calmodulin binding to eNOS in the regulation of enzyme activity.21 Paralleling this typical mode of agonist activation, the stimulation of the enzyme by HDL entails its phosphorylation at Ser1179 via Akt, and this is mediated by Src family kinases and PI3 kinase. Enzyme activation also requires Src-dependent and PI3 kinase–dependent activation of Erk1/2 MAP kinases.90 Paralleling these findings in cell culture, there is increased Akt and Erk1/2 phosphorylation in the aortas of apoA-I transgenic mice and decreased abundance of the phosphorylated proteins in the aortas of apoA-I−/− mice.91 It has been shown using calcium chelation and other approaches that changes in intracellular calcium homeostasis in endothelial cells are required for NO formation in response to HDL.92–94

Role of HDL-Associated Molecules and HDL Modifications

Several studies have indicated that lysophospholipids associated with HDL participate in eNOS activation. For example, native HDL and the potential HDL cargos sphingosylphosphorylcholine, S1P, and lysosulfatide cause parallel eNOS-dependent relaxation of precontracted aortic rings from mice, and 50% to 60% of the response to native HDL is lost in rings from mice lacking the lysophospholipid receptor S1P₂, which mediates responses to S1P and lysosulfatide.95 Studies in cell culture have shown that the receptor for sphingosylphosphorylcholine in endothelial cells is G protein-coupled receptor 4,95 but loss-of-function studies of HDL actions in the absence of G protein-coupled receptor 4 have not yet been pursued. However, the intravenous administration of HDL stimulates myocardial perfusion in vivo equivalently in wild-type and S1P₂−/− mice but not in eNOS−/− mice.96 As such, the role of eNOS in HDL-induced vascular responses remains clear, whereas the involvement of lysophospholipids as physiologically HDL-associated eNOS agonists may vary between endothelium from different vascular sources.

Prompted by recent findings of attenuated endothelial actions of HDL isolated from patients with coronary artery disease or acute coronary syndrome versus HDL from healthy controls, it has been discovered that malondialdehyde (MDA) associated with HDL can impact the ability of the lipoprotein to activate eNOS. It was found that MDA content is elevated in HDL from coronary disease patients compared with HDL from healthy subjects, that there is a parallel diminution in the capacity of the HDL from the former group to activate eNOS, and that the addition of MDA to HDL from healthy individuals blunts endothelial NO production. The antagonistic action of MDA was determined to be mediated by lectin-type oxidized LDL receptor 1 activation of protein kinase C-βII, which inhibits Akt-activating phosphorylation (Akt-Ser473) and eNOS-activating phosphorylation at Ser1177 (in human eNOS). Because MDA formation is decreased by HDL-associated paraoxonase 1 (PON1),97 PON1 activity was evaluated and was found to be markedly decreased in HDL from coronary disease patients. Furthermore, PON1 inactivation in HDL from healthy subjects results in greater protein kinase C-βII activation in cultured endothelial cells, decreased activating eNOS-Ser1177 phosphorylation, and increased inactivating eNOS-Thr495 phosphorylation, resulting in attenuated NO production. Furthermore, HDL from PON1−/− mice fails to stimulate endothelial cell NO production, and the supplementation of PON1−/− HDL with purified PON1 restores this function.98 These collective observations indicate that HDL-associated MDA and PON1 may have major impact on endothelial function, and the latter is consistent with the reported inverse relationship between PON1 activity and cardiovascular disease development.99

In addition to potentially important influences of HDL-associated molecules, oxidative modification of HDL alters its capacity to activate eNOS. Myeloperoxidase-mediated modifications of apoA-I were first recognized to impair HDL-mediated cholesterol efflux from macrophages,100 and serum myeloperoxidase levels predict the risk of coronary artery disease and are independently associated with endothelial dysfunction.101,102 These observations prompted studies of the impact of HDL oxidation on the ability of the lipoprotein to stimulate eNOS. The exposure of HDL from healthy subjects
to myeloperoxidase-derived oxidants that were either nitrating or chlorinating oxidant species caused complete prevention of eNOS activation. Therefore, the characteristics of HDL that influence its ability to stimulate eNOS enzymatic activity are multiple.

Impact on eNOS Protein Abundance
In addition to the regulation of NO production by signaling events that modulate eNOS enzymatic activity, important control of eNOS involves changes in the abundance of the enzyme. In cultured human endothelial cells, eNOS protein expression is increased three-fold by HDL exposure for 24 hours, and the increase in eNOS protein is not associated with an increase in steady-state mRNA levels. Alternatively, it is related to a comparable three-fold increase in the half-life of the protein, which is mediated by PI3 kinase/Akt and MAP kinases, indicating that the same signaling mechanisms that underlie the acute activation of eNOS by HDL are operative in upregulating the abundance of the enzyme as well. An upregulation in eNOS protein abundance in response to HDL also has been observed in EPC. However, whether HDL influences eNOS enzyme abundance in vivo remains unknown.

Modulation of eNOS in Humans
A relationship between HDL and endothelium-dependent vasodilation has been known since 1994, when it was reported that patients with elevated HDL have greater vasodilator and attenuated vasoconstrictor response. Subsequent studies of flow-mediated vasodilation of the brachial artery revealed that HDL cholesterol is an independent predictor of endothelial function. In an interventional study of HDL elevation by niacin treatment for 3 months in patients with coronary artery disease and decreased HDL levels, niacin administration and the resulting 25% increase in HDL were associated with a marked improvement in flow-mediated vasodilation.

The direct, short-term impact of HDL on endothelial function also has been investigated in humans. One study analyzed forearm blood flow responses in ABCA1 heterozygotes. Compared with controls, ABCA-1 heterozygotes (six men and three women) had HDL levels that were decreased by 60%, and their blood flow responses to endothelium-dependent vasodilators were blunted despite their having normal endothelium-independent responses. After a 4-hour infusion of apoA-I/phosphatidylcholine disks, HDL levels in the ABCA1 heterozygotes increased three-fold, and endothelium-dependent vasomotor responses were normalized.

Indirect Vascular Actions of HDL
Modulation of Glucose Homeostasis
In addition to its direct actions on cells that govern vascular health and disease, HDL may favorably influence glucose homeostasis and thereby has potentially novel functions that indirectly promote vascular health. ApoA-I/HDL stimulates glucose uptake by skeletal muscle myocytes via increasing AMP-activated protein kinase activity; it also promotes glycogen synthesis by skeletal muscle myocytes, and via SR-BI it stimulates glucose uptake by adipocytes. HDL also promotes insulin secretion by pancreatic β cells. The latter process requires ABCA1 when lipid-free apoA-I is the stimulus and requires ABCG1 when recombinant HDL is the stimulus; with either lipid-free apoA-I or recombinant HDL, pancreatic β-cell SR-BI is required. Mice with pancreatic β-cell-specific deletion of ABCA1 display glucose intolerance, elegantly demonstrating that these mechanisms are operative in vivo. Furthermore, whereas VLDL and LDL particles attenuate insulin mRNA levels and β-cell proliferation and cause β-cell apoptosis, HDL antagonizes the proapoptotic signaling by VLDL and LDL in an Akt-dependent manner. The impact of HDL on glucose homeostasis also has been demonstrated in individuals with type 2 diabetes mellitus, with HDL administration resulting in a lowering of plasma glucose, increases in insulin levels, and increased AMP-activated protein kinase activity in skeletal muscle.

Modulation of Adipose
Mouse models have shown that in addition to influencing glucose homeostasis, apoA-I and HDL potentially combat obesity, with the overexpression of apoA-I or the administration of the apoA-I mimetic D-4F decreasing white adipose mass and insulin resistance and increasing energy expenditure in mice fed a high-fat diet. Brown adipose tissue was found to be another potential target tissue of apoA-I and HDL action because the overexpression of apoA-I increased uncoupling protein 1 mRNA abundance in brown adipose in vivo, and parallel findings were made in brown adipocytes in culture. In ob/ob mice, the apo-A-I mimetic L-4F lowers adiposity and inflammation and causes an improvement in glucose tolerance. In addition, apoA-I gene transfer in mice causes an increase in plasma concentrations of the adipokine adiponectin and an increase in adiponectin expression in abdominal fat, and HDL treatment of cultured adipocytes promotes adiponectin expression in a PI3 kinase–dependent manner. Thus, HDL influences the production of an adipose-derived cytokine that itself affords vascular protection. Furthermore, in patients with type 2 diabetes mellitus, the administration of rHDL inhibits fasting-induced lipolysis and oxidation, suggesting additional impact of HDL on lipid homeostasis. Interestingly, there is also in vitro and in vivo evidence that adipocytes, which are a potentially large source of free cholesterol, support the transfer of cholesterol to HDL via ABCA1 and SR-BI, indicating potential reciprocal modes of regulation between the lipoprotein and adipose.

Molecular Basis of HDL Signaling
As outlined, HDL induces a variety of signaling events that underlie numerous actions of the lipoprotein in target cells. The molecular basis of HDL signaling that occurs independent of cargo molecules has been investigated by interrogation of the proximal mechanisms in HDL activation of eNOS. In cultured endothelial cells, short-term exposure to HDL or methyl-β-cyclodextrin causes eNOS stimulation of similar magnitude, whereas cholesterol-loaded methyl-β-cyclodextrin does not. Cholesterol-free Lp2A-I...
particles composed of lipid-free recombinant apoA-I and phosphatidylcholine also activate eNOS, whereas cholesterol containing LpA-I particles do not. In addition, phosphatidylycerol-choline-loaded HDL causes greater eNOS stimulation than native HDL, and blocking antibody to SR-BI, which retards cholesterol efflux, prevents eNOS activation. Furthermore, in a reconstitution system in COS-M6 cells, wild-type SR-BI mediates eNOS activation by both HDL and small unilamellar vesicles, whereas the SR-BI mutant AVI, which is incapable of efflux to small unilamellar vesicles, transmits signal only in response to HDL. Furthermore, eNOS activation by both HDL and methyl-β-cyclodextrin is SR-BI-dependent. The capacity of methyl-β-cyclodextrin to invoke cholesterol efflux is not mediated by SR-BI or any other cell surface protein, these cumulative findings in the context of eNOS regulation indicate that signal initiation by HDL requires cholesterol efflux, that the apolipoprotein and phospholipid components of HDL are sufficient to initiate signaling, and that SR-BI may serve as a sensor of cholesterol movement in the plasma membrane.

The participation of cholesterol efflux in HDL action also has been evaluated in nonendothelial cells and processes other than eNOS regulation. In monocytes, HDL and methyl-β-cyclodextrin cause equal antagonism of CD11b expression, which contributes to HDL attenuation of monocyte–endothelial cell adhesion, whereas cholesterol-laden methyl-β-cyclodextrin has no effect. In pancreatic β cells, insulin secretion in response to discoidal apoA-I recombinant HDL is absent after ABCG1 knockdown; paralleling the actions of native HDL on β cells, there is an initiation of insulin secretion within 10 minutes of treatment with methyl-β-cyclodextrin, even in the absence of other insulin secretagogues. Thus, cholesterol efflux may be mechanistically involved in a variety of actions of HDL in diverse target cells.

The features of SR-BI required for signal initiation also have been interrogated. Using SR-BII, which is a splice variant of SR-BI, and mutant and chimeric class B scavenger receptors, it was determined that the C-terminal cytoplasmic PDZ-interacting domain and the C-terminal transmembrane domain of SR-BI are both required for HDL signaling. In addition, in studies using a photoactivated derivative of cholesterol, direct binding of cholesterol to the C-terminal transmembrane domain was demonstrated. Thus, key domains of SR-BI uniquely mediating HDL signaling have been initially identified.

Assessments of HDL Function

During the past 10 years, the recognition that variance in plasma HDL cholesterol concentration may not be associated with differences in cardiovascular disease risk and that HDL has potentially important cardiovascular-protective functions other than its participation in RCT has prompted comparisons of HDL function in different patient populations. In studies evaluating the capacity of HDL to blunt LDL-induced monocyte chemotaxis in a human artery coculture system, it was found that whereas the lipoprotein isolated from control subjects effectively inhibited chemotaxis, HDL from coronary heart disease patients with either normal or even elevated circulating HDL levels lacked this function.

investigations of the ability of HDL to reverse the inhibition of aortic ring endothelium-dependent relaxation by oxLDL, HDL isolated from subjects with type 1 or type 2 diabetes mellitus or abdominal obesity had far less effect than HDL prepared from healthy control subjects. It also has been observed that whereas HDL from healthy controls stimulates endothelial NO production, EPC-mediated repair, and endothelium-dependent vasodilation, these actions are impaired in HDL obtained from patients with type 2 diabetes mellitus. The endothelial actions of HDL have been further interrogated in a recent investigation of lipoprotein isolated from patients with stable coronary artery disease or acute coronary syndrome versus that from healthy individuals. The HDL from controls caused an increase in bioavailable NO in endothelial cells and resulted in promotion of endothelial repair and an attenuation of nuclear factor-xB activation and vascular cell adhesion molecule 1 expression, thereby preventing endothelial cell–monocyte adhesion; in contrast, the HDL from coronary disease patients lacked these properties and was found to be related to elevated MDA content that was further attributed to a decrease in HDL-associated PON1 activity. Thus, valuable information about HDL functions beyond those involved in the processes in RCT in different patient populations is emerging.

Along with learning more about the functions of HDL in various patient groups, changes in HDL action in response to therapies targeting the lipoprotein also have been observed. In addition to increasing HDL cholesterol plasma levels in patients with type 2 diabetes mellitus, extended-release niacin therapy was found to cause improvements in the endothelial protective functions of the lipoprotein. Treatment with the cholesteryl ester transfer protein inhibitor torcetrapib resulted in a modest increase in the capacity of HDL to invoke net cholesterol efflux from foam cells, mirroring the enhanced efflux capacity of HDL from individuals with cholesteryl ester transfer protein deficiency. Through continuing accumulation of observations of this type, assessments of HDL function comparing different patient populations and assessing changes with therapeutic intervention will enhance our understanding of the novel actions of the lipoprotein.

Current Knowledge Gaps

Our present understanding of the cellular targets of HDL action and the mechanisms by which the lipoprotein initiates intracellular signaling are summarized in Figures 1 and 2, respectively. The current knowledge gaps in this realm of HDL biology are multiple. First, we do not know which proximal processes invoked by HDL in various target cells are shared among the different cell types and functional responses and which are unique. Cholesterol efflux by HDL is important in eNOS activation in endothelial cells and likely participates in anti-inflammatory processes in HDL-exposed monocytes and the capacity of HDL to promote pancreatic β-cell insulin secretion. However, whether cholesterol efflux is a uniform mechanism whereby HDL initiates intracellular signaling in other HDL target cells warrants elucidation. Second, although we have gained a reasonable understanding of the initial intracellular signaling events such as kinase cascade activation that...
may be prompted by HDL in various cell types, we have a meager understanding of the genes whose expression is modified by HDL in its various target cells. Third, we do not know to what extent each of the many functions of HDL that we have discussed in this review impacts cardiovascular and metabolic health. Fourth, we do not know whether assays of HDL action on endothelial cells or leukocytes or VSM cells, for example, will increase our ability to assign cardiovascular disease risk, or whether they will enhance our understanding of the outcomes of future trials testing HDL-targeted therapies. Finally, remembering that HDL particles are composed of approximately 100 different proteins and a large variety of lipid species and that HDL subclasses differ in their numerous physical and chemical properties,135

Figure 1. Cell targets of high-density lipoprotein (HDL) action. ROS indicates reactive oxygen species. (Illustration credit: Ben Smith.)

Figure 2. Mechanisms of high-density lipoprotein (HDL) signal initiation. Intracellular signaling in HDL target cells is initiated by a variety of mechanisms. Numerous responses require apolipoprotein A-I (apoA-I) binding to scavenger receptor, class B, type I (SR-BI), and certain forms of intracellular signaling occur in response to cholesterol efflux to HDL (orange circles). The HDL-associated lysophospholipid sphingosylphosphorylcholine (SPC) activates G protein-coupled receptor 4 (GPR4) and sphingosine-1-phosphate (S1P) and lysosulfatide (LSF) initiate intracellular signaling via S1P1,3. HDL-associated malondialdehyde (MDA) modifies intracellular signaling via lectin-type oxidized low-density lipoprotein (LDL) receptor 1 (LOX-1), and paraoxonase 1 (PON1) associated with HDL tempers MDA formation. (Illustration credit: Ben Smith.)
we currently have a modest understanding of how most of these features influence the functions of the lipoprotein and therefore the cellular responses to the lipoprotein. What we now know, however, is that the influence of HDL on cardiovascular and metabolic health likely goes well beyond what we now know, however, is that the influence of HDL on those functions and the cellular responses to the lipoprotein. What we currently have a modest understanding of how most of those features influence the functions of the lipoprotein and therefore the cellular responses to the lipoprotein.

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Disclosures

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