Thematic Review Series: High Density Lipoprotein Structure, Function, and Metabolism

Regulation of signal transduction by HDL[1]

Chieko Mineo and Philip W. Shaul[2]

Division of Pulmonary and Vascular Biology, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX

Abstract High density lipoprotein (HDL) cholesterol has direct effects on numerous cell types that influence cardiovascular and metabolic health. These include endothelial cells, vascular smooth-muscle cells, leukocytes, platelets, adipocytes, skeletal muscle myocytes, and pancreatic β cells. The effects of HDL or apoA-I, its major apolipoprotein, occur through the modulation of intracellular calcium, oxygen-derived free-radical production, numerous kinases, and enzymes, including endothelial nitric-oxide synthase (eNOS). ApoA-I and HDL also influence gene expression, particularly genes encoding mediators of inflammation in vascular cells. In many paradigms, the change in intracellular signaling occurs as a result of cholesterol efflux, with the cholesterol acceptor methyl-β-cyclodextrin often invoking responses identical to HDL or apoA-I. The ABC transporters ABCA1 and ABCG1 and scavenger receptor class B, type I (SR-BI) frequently participate in the cellular responses. Structure-function relationships are emerging for signal initiation by ABCA1 and SR-BI, with plasma membrane cholesterol binding by the C-terminal transmembrane domain of SR-BI uniquely enabling it to serve as a sensor of changes in membrane cholesterol.■■ Further investigation of the processes underlying HDL and apoA-I modulation of intracellular signaling will potentially reveal new prophylactic and therapeutic strategies to optimize both cardiovascular and metabolic health.—Mineo, C., and P. W. Shaul. **Regulation of signal transduction by HDL.** *J. Lipid Res.* **2013.** 54: 2315–2324.

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Our understanding of how high density lipoprotein (HDL) cholesterol potentially modifies cardiovascular and metabolic disease risk or outcome has expanded beyond its participation in reverse cholesterol transport (RCT), in which HDL serves to shuttle cholesterol from peripheral tissues or cells to the liver. This review highlights recent advances in our knowledge of HDL-initiated processes mediated by changes in intracellular signaling in numerous cell types of relevance to both cardiovascular and metabolic conditions, which represent actions of the lipoprotein beyond its classical role in global cholesterol homeostasis. The evidence that HDL influences cardiovascular and metabolic health and disease will first be briefly summarized. Responses to HDL or to apoA-I, its major apolipoprotein, in cellular targets directly involved in vascular biology will then be reviewed, followed by a summary of the mechanisms that HDL influences in cell types participating in energy and glucose homeostasis. The processes underlying apoA-I- or HDL-induced changes in intracellular signaling will then be discussed, and finally presently unanswered questions in this realm of HDL biology will be considered. Although HDL cargo molecules can contribute to cellular responses to the lipoprotein (1, 2), herein emphasis will be placed primarily on signaling events directly induced by apoA-I or HDL, which are likely occurring in response to cholesterol efflux. To help leverage our present understanding of apoA-I- or HDL-initiated signaling in future studies, the signaling events and the proteins or mediators whose abundance or activity is altered by apoA-I or HDL in various cell types are summarized in **Tables 1** and **2**.

Abbreviations: AMPK, AMP-activated protein kinase; CaMKK, calcium/calmodulin-dependent protein kinase kinase; COX-2, cyclooxygenase type 2; DHCR24, 3β-hydroxysteriod-Δ24 reductase; eNOS, endothelial nitric-oxide synthase; EPC, endothelial progenitor cell; GKS3, glycogen synthase kinase 3; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule-1; JAK2, Janus kinase 2; LKB1, liver kinase B1; MCP-1, monocyte chemoattractant protein-1; PKA, protein kinase A; PKC, protein kinase C; RCT, reverse cholesterol transport; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SAA3, serum amyloid A3; SR-BI, scavenger receptor class B, type I; VCAM-1, vascular cell adhesion molecule-1; VSM, vascular smooth muscle.

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[2] To whom correspondence should be addressed.
  e-mail: philip.shaul@utsouthwestern.edu

TABLE 1. Signaling modulated by apoA-I or HDL

Process or Signaling Molecule	Cell Type	Reference
Intracellular calcium	Endothelium, platelet, macrophage	(46), (69), (70), (71), (88), (100)
Oxygen-derived free radicals	Endothelium, VSM, adipocyte	(24), (48), (92)
eNOS	Endothelium, EPC	(57), (64)
Akt kinase	Endothelium, EPC, adipocyte, β cell	(37), (48), (58), (67), (94)
PI3 kinase	Endothelium, EPC, adipocyte	(49), (56), (58), (67), (91)
G proteins	Endothelium	(49), (55)
p38 MAP kinase	Endothelium, platelet	(49), (87)
Rho kinase	Endothelium, fibroblast	(49), (84), (108)
Ras	Endothelium	(55)
p42/44 MAP kinase	Endothelium, EPC	(57), (67)
Rac GTPase	Endothelium, VSM	(24), (56)
Src kinase	Endothelium	(56), (67)
AMPK	Endothelium, skeletal muscle, adipocyte	(35), (37), (62)
CaMKK	Endothelium	(62)
LKB1	Endothelium	(62)
JAK2	Macrophage	(104)
STAT3	Macrophage	(104)
GSK3	Skeletal muscle	(37)
Adenylate cyclase	Fibroblast	(99)
Cdc42	Fibroblast	(105)

HDL AND CARDIOVASCULAR AND METABOLIC DISEASE

Studies of the relationship between circulating concentrations of HDL and atherosclerosis and cardiovascular events have suggested that disease risk is inversely related to HDL level (3, 4). Even in patients treated aggressively with statins to decrease circulating low-density lipoprotein (LDL) cholesterol to less than 70 mg/dl, HDL levels may continue to be inversely related to the risk of major cardiovascular events (5). However, genetic analyses of mechanisms that regulate plasma HDL have yielded conflicting results regarding the relationship between HDL abundance and disease risk (6, 7), and the findings of studies of interventions aimed at raising HDL have also been mixed (5, 8–11). From a mechanistic perspective, HDL classically functions in RCT, removing cholesterol from peripheral tissues and cells, such as macrophages, and delivering it to the liver and to steroidogenic organs by binding of apoA-I to the high-affinity HDL receptor scavenger receptor, class B, type I (SR-BI) (12, 13). In mouse models of atherosclerosis, both apoA-I and SR-BI provide atheroprotection (14, 15), and the provision of apoA-I or HDL also attenuates neointima formation after artery injury in the context of experimental hypercholesterolemia (16, 17). The potential protective nature of HDL has been principally attributed to its ability to promote RCT by accepting cholesterol from lipid-laden macrophages (18, 19). However, there are other actions of the lipoprotein likely relevant to cardiovascular protection, including direct effects on endothelium (20), vascular smooth muscle (21–24), leukocytes (25, 26), and platelets (27, 28). Evaluations of HDL function have received considerable attention recently as potential means to interrogate the characteristics

of the lipoprotein and how they relate to cardiovascular disease risk and severity. These include assessments of cholesterol efflux capacity from macrophages (29, 30) and direct actions of HDL on endothelium (31, 32).

In addition to its relationship with atherosclerosis and cardiovascular events, there is a recognized association between HDL and insulin sensitivity, with low HDL levels and dysfunctional HDL associated with insulin resistance and type 2 diabetes (33). Possible effects of HDL on glucose regulation are evident from studies in humans as well as experimental models. The administration of reconstituted HDL to patients with type 2 diabetes causes a fall in plasma glucose (34), and apoA-I$^{-/-}$ mice have fasting hyperglycemia and hyperinsulinemia and abnormal glucose tolerance tests (35). In addition, HDL directly modifies the functions of cell types involved in glucose homeostasis, including pancreatic β cells (36), skeletal muscle myocytes (35, 37), and adipocytes (37). It has also been found that apoA-I has antiobesity effects, with transgenic mice overexpressing apoA-I and mice administered apoA-I mimetic D-4F demonstrating protection from increased adiposity caused by high-fat diet feeding (38). In ob/ob mice, the apoA-I mimetic L-4F attenuates adiposity and causes an improvement in glucose tolerance (39). Conversely, type 2 diabetes and its associated hyperlipidemia and hyperglycemia negatively impact not only HDL abundance but also HDL quality, resulting in more glycated and triglyceride-rich HDL (40, 41). Mirroring the recent interest in evaluating HDL function in subjects with cardiovascular disease, the cellular actions of HDL isolated from diabetic and obese individuals have been studied (42–45). In the midst of attempts to better understand how HDL impacts both cardiovascular and metabolic health and how HDL function may be altered in various patient populations, diverse intracellular signaling events modulated by apoA-I or the lipoprotein have been identified.

HDL SIGNALING IN VASCULAR CELLS

Endothelial cells

Studies of the direct impact of HDL on endothelial cell apoptosis were some of the first to indicate that the lipoprotein alters intracellular signaling in endothelium. Oxidized LDL (OxLDL) causes a delayed but sustained increase in intracellular calcium in endothelial cells that results in cell death, and this is reversed by HDL via prevention of the increase in intracellular calcium. Purified apoA-I provides the same protection as native HDL, and it requires HDL binding to the cells and new protein synthesis (46). Tumor necrosis factor-α (TNF-α)-induced endothelial cell apoptosis is also inhibited by HDL, and this is associated with diminished induction of caspase 3, which is a component of all primary apoptotic pathways (47). HDL also attenuates growth factor deprivation-related apoptosis of endothelial cells. This is due to blunting of the mitochondrial pathway of apoptosis, with HDL diminishing the dissipation of mitochondrial potential, oxygen-derived

TABLE 2. Protein or mediator abundance or activity modulated by apoA-I or HDL

Protein or Mediator	Cell Type	Reference
Caspase 3 and 9 (downregulation)	Endothelium, β cell	(47), (48), (94)
VCAM-1 (downregulation)	Endothelium	(59), (60)
ICAM-1 (downregulation)	Endothelium	(59), (60)
DHCR24 (upregulation)	Endothelium	(61)
HO-1 (upregulation)	Endothelium	(61)
E-selectin (downregulation)	Endothelium	(59)
eNOS (upregulation)	Endothelium, EPC	(72), (73)
COX-2 (upregulation)	VSM	(21)
MCP-1 (downregulation)	VSM, adipocyte	(24), (92)
CD11b (downregulation)	Monocyte, neutrophil	(25), (26)
Cytokines (downregulation)	Monocyte, macrophage	(74), (76)
NF-kB (downregulation)	Endothelium	(63)
Prostacyclin (upregulation)	Endothelium	(81)
Platelet-activating factor (downregulation)	Endothelium	(82)
Thromboxane A2 (downregulation)	Endothelium	(83)
Tissue factor (downregulation)	Endothelium	(84)
SOD1, SOD2 (upregulation)	Macrophage	(77)
Nox2 (downregulation)	Macrophage	(77)
Uncoupling protein I (upregulation)	Brown adipocyte	(38)
Adiponectin (upregulation)	Adipocyte	(91)
SAA3 (downregulation)	Adipocyte	(92)

free-radical generation, cytochrome c release to the cytoplasm, and caspases 3 and 9 activation. HDL also activates Akt and causes phosphorylation of the Akt target BAD, which favors BAD disassociation from BCL-X$_L$ that is then free to inhibit mitochondria-mediated apoptosis. The HDL-associated lysophospholipids sphingosylphosphorylcholine (SPC) and lysosulfatide (LSF) protect endothelial cells from growth factor deprivation-related apoptosis via mechanisms paralleling those of native HDL (48). In addition, the lysophospholipid sphingosine-1-phosphate (S1P) enhances endothelial cell survival with effects comparable to those of native HDL, and these responses are inhibited by knockdown of the S1P receptor EDG-1/S1P$_1$, by pertussis toxin, and by PI3 kinase and Erk pathway antagonists, suggesting that signaling by lysophospholipid components of HDL may be important for the inhibition of apoptosis (49). Although the vast 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, the mimetic improved vascular reactivity and decreased endothelial cell fragmentation and sloughing (50), suggesting that the antiapoptotic actions of HDL may be operative in vivo.

In addition to its antiapoptotic actions on endothelial cells, it was recognized over 30 years ago that HDL directly stimulates endothelial cell proliferation (51, 52) and that this occurs through calcium-dependent processes (53). In 1994 it was further reported that HDL stimulates endothelial cell migration independent of cell proliferation (54). The basis for the migration response has been somewhat controversial, with some reports indicating that pertussis toxin inhibits the response and others showing no effect of pertussis toxin (49, 54). The former studies further

implicated the G protein-coupled S1P receptors EDG-1/S1P$_1$ and EDG-3/S1P$_3$ and the S1P-rich fraction of HDL, and dependence on PI3 kinase, p38 MAP kinase, and Rho kinase (49). Capillary tube formation stimulated by HDL in vitro has been found to be pertussis toxin-sensitive but independent of p38 MAP kinase, alternatively requiring p42/44 MAP kinase activity residing downstream of Ras (55). It has also been observed that HDL stimulates endothelial cell migration in vitro via the activation of Rac GTPase; this process does not require HDL cargo molecules, and it is dependent on SR-BI and the activation of Src kinases, PI3-kinase, and p44/42 MAP kinases. Rapid initial stimulation of lamellipodia formation by the HDL/SR-BI tandem via Src kinases and Rac also occurs in cultured endothelial cells (56). Considering that few experiments have been done in vivo, the inconsistences in the implicated intracellular signaling events may relate to the diversity of endothelial cell types employed in cell culture studies and possibly also variance in the experimental conditions used. Along with promoting growth and migration and tube formation by differentiated endothelial cells, HDL action via SR-BI activates the same processes in endothelial progenitor cells (EPC). The responses in EPC are dependent on PI3 kinase, Akt, p42/44 MAP kinase, and endothelial nitric-oxide synthase (eNOS) (57, 58).

A third category of direct impact of HDL on endothelium involves its anti-inflammatory actions. In particular, HDL attenuates the expression of the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin in cultured endothelial cells. This process is mediated by SR-BI and PI3 kinase and eNOS, and in some studies, also by S1P receptors, in the latter case implicating the involvement of HDL-associated S1P (59, 60). There is also evidence that lipid-free apoA-I and recombinant HDL inhibit endothelial cell adhesion molecule expression by upregulating the expression of 3β-hydroxysteriod-Δ24 reductase (DHCR24) and heme oxygenase-1 (HO-1), with the increased expression by HO-1 being mediated by DHCR24 upregulation and resulting activation of PI3 kinase and Akt (61). Other studies have indicated that HDL activation of AMP-activated protein kinase (AMPK) is required in HDL modulation of adhesion molecule expression and that the signaling events proximal to AMPK involve calcium/calmodulin-dependent protein kinase kinase (CaMKK) and liver kinase B1 (LKB1) (62). Recognizing the key role of NF-κB in the modulation of cellular responses to inflammation, it has also been shown that apoA-I decreases NF-κB activation induced by palmitate in cultured endothelial cells (63).

The capacity of HDL to activate eNOS, which was first described in 2001, may be the primary signaling mechanism that commonly underlies the capacity of the lipoprotein both to have antiapoptotic and anti-inflammatory action in endothelial cells and to promote their proliferation and migration (64). In cultured endothelial cells, potential apoA-I-eNOS interaction and perinuclear colocalization have been reported (65). 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, where eNOS is localized in caveolae/lipid rafts (66), 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 (64). The stimulation of eNOS enzymatic activity by HDL entails eNOS phosphorylation at Ser1179 via Akt, and this is mediated by Src family kinases and PI3 kinase. Enzyme activation by HDL also requires Src- and PI3 kinase-dependent activation of Erk1/2 MAP kinases (67). 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 (68). It has further been shown using calcium chelation and other approaches that changes in intracellular calcium homeostasis in endothelial cells are required for nitric-oxide formation in response to HDL (69–71). Potentially further amplifying the impact of HDL on the capacity for nitric-oxide production by endothelial cells, the lipoprotein also increases eNOS protein abundance, not by altering gene transcription but by increasing the protein half-life through PI3 kinase, Akt, and p42/44 MAP kinase-dependent processes (72). HDL upregulation of eNOS protein has also been demonstrated in EPC (73).

Vascular smooth-muscle cells

HDL has numerous direct effects on intracellular signaling in vascular smooth-muscle (VSM) cells that are potentially important to the modulation of VSM function by the lipoprotein. HDL enhances VSM cell prostacyclin production, which results from the upregulation of cyclooxygenase type 2 (COX-2) expression (21). HDL also inhibits VSM cell migration via S1P-mediated processes (22, 23). Through its S1P cargo, HDL additionally blunts the expression of monocyte chemoattractant protein-1 (MCP-1) in VSM cells, and this is mediated by the S1P$_3$ receptor for S1P, and an attenuation of reactive oxygen species (ROS) production, which regulates MCP-1 production. The influence of HDL on ROS production in VSM is through prevention of the activation of Rac1 and resulting inhibition of NAD(P)H oxidase (24).

Leukocytes

In addition to its anti-inflammatory actions on endothelial cells, HDL directly blunts monocytes/macrophage and neutrophil activation (25, 26). In monocytes, both native HDL and apoA-I dampen phorbol 12-myristate 13-acetate (PMA) induction of the integrin CD11b, which promotes adhesion and migration, thereby attenuating PMA-related enhancement of monocyte-endothelial cell interaction (26). HDL also inhibits the binding of T-cell microparticles to monocytes, resulting in a diminution in proinflammatory cytokine production (74). ApoA-I also attenuates dendritic cell differentiation from monocytes (75). In macrophages, apoA-I actions mediated by Janus kinase 2

(JAK2) activation of STAT3 diminish the induction of inflammatory cytokines by LPS (76). Lipid-free apoA-I, reconstituted HDL, and native HDL also inhibit high-glucose-induced redox signaling in macrophages, with lipid-free apoA-I doing so through ABCG1-dependent increases in superoxide dismutase 1 and superoxide dismutase 2, and an attenuation in Nox2 expression (77). In neutrophils, native HDL and apoA-I decrease the surface expression of CD11b. In parallel, HDL and apoA-I decrease neutrophil spreading and migration and neutrophil-platelet interaction (25). HDL additionally inhibits the proliferation of hematopoietic stem cells and multipotential progenitor cells. Regarding the potential underlying signaling events, in bone marrow-derived cells HDL blunts Erk1,2 activation and Ras plasma membrane recruitment and activation by either IL-3 or GM-CSF (78).

Platelets

Studies in both humans and animal models indicate that HDL inhibits platelet activation (28, 79, 80). At least a portion of the underlying mechanisms in vivo are indirect, occurring via actions of HDL on the endothelium that include not only the activation of endothelial nitric oxide and prostacyclin production (64, 81) but also the downregulation of platelet-activating factor (82), thromboxane A2 (83), and tissue factor production by endothelium. The attenuation of endothelial cell tissue factor expression by HDL involves the inhibition of RhoA and activation of PI3 kinase (84). There is also evidence that HDL has direct action on platelets (27, 28). Platelet aggregation stimulated by a variety of means, including by thrombin and ADP, is reduced by HDL, and the lipoprotein also decreases thromboxane A2 and 12-hydroxy-eicosatetraenoic acid release from platelets (85, 86). HDL activates p38MAPK in platelets (87), and it attenuates intracellular calcium mobilization invoked by LDL cholesterol (88). ApoA-I-rich HDL may exert its actions on platelets via SR-BI, whereas responses to apoE-rich HDL are likely mediated by a splice variant of the LDL receptor family member apoER2 or Lrp8 (85, 89).

HDL SIGNALING AND METABOLISM

Adipocytes

HDL promotes glucose uptake by adipocytes via processes that require SR-BI and possibly also Akt and AMPK activation (37). In brown adipocytes, apoA-I upregulates the expression of uncoupling protein I (38), which participates in the control of energy expenditure (90). In addition, in cultured adipocytes via PI3 kinase-dependent processes, HDL upregulates the expression of adiponectin, which is an adipokine that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation, and also has cardiovascular-protective actions (91). In adipocytes, apoA-I and HDL also have anti-inflammatory action, blunting MCP-1 and serum amyloid A3 (SAA3) expression and diminishing the translocation

of NADPH oxidase 4 into lipid rafts, thereby attenuating ROS generation. These responses are dependent on ABCA1, ABCG1, and SR-BI (92).

Skeletal muscle myocytes

ApoA-I/HDL stimulates glucose uptake in skeletal muscle myocytes via increasing AMPK activity (35). Using L6 myotubes and myofibers studied ex vivo, it has been found that the effect of reconstituted HDL on glucose uptake is comparable to that observed with insulin, and that it is associated with increased plasma membrane GLUT4 (93). ApoA-I/HDL also promotes glycogen synthesis in myocytes in association with increases in glycogen synthase kinase 3 (GKS3) phosphorylation (37).

Pancreatic beta cells

HDL promotes insulin secretion by pancreatic β cells through processes that involve ABCA1 when lipid-free apoA-I is the stimulus, and through ABCG1-dependent mechanisms when the stimulus is recombinant HDL. With either stimulus, SR-BI is required (36). Via the activation of Akt, HDL also counteracts the proapoptotic actions of VLDL or LDL in β cells (94).

HDL SIGNALING MECHANISMS

HDL signaling and cholesterol efflux

As outlined above, HDL induces a variety of signaling events that underlie numerous actions of the lipoprotein in target cells (95, 96). 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 Lp2A-I particles do not. In addition, phosphatidylcholine-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. Moreover, eNOS activation in response to either HDL or methyl-β-cyclodextrin is SR-BI dependent (97). Since 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 provided the first indication 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 has also been evaluated in nonendothelial cells and in processes besides 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 (26). In pancreatic β cells, insulin secretion in response to discoidal apoA-I-recombinant HDL is absent following ABCG1 knockdown (36), and paralleling the actions of native HDL on β cells, there is an initiation of insulin secretion within 10 min of treatment with methyl-β-cyclodextrin, even in the absence of other insulin secretagogues (98). In adipocytes, the capacity of apoA-I and HDL to decrease MCP-1 expression and NADPH oxidase 4 plasma membrane translocation, which underlies NADPH oxidase 4 promotion of ROS generation, is associated with lowering of plasma membrane cholesterol content. In addition, these responses are mimicked by methyl-β-cyclodextrin, and they are dependent on ABCA1, ABCG1, and SR-BI (92). These collective findings indicate that cholesterol efflux may be mechanistically involved in a variety of the actions of HDL in diverse target cells.

ABCA1

As mentioned above, the adenosine triphosphate-binding cassette (ABC) transporters ABCA1 and ABCG1, which are classically involved in cholesterol efflux to apoA-I and in nascent HDL particle assembly, have been implicated in certain cellular responses to apoA-I and HDL. ABCA1 mediates the direct effects of apoA-I in neutrophils (25) and monocytes (26), and ABCA1 and ABCG1 both participate in the actions of HDL in hematopoietic stem cells (78), pancreatic β cells (36), and adipocytes (92).

In addition to regulating the primary functions of these various cell types, such as inflammation-related processes in neutrophils, there is evidence that signaling invoked by ABCA1 impacts its abundance and its capacity to mediate lipid transport to apoA-I. Via coupling to G_s, apoA-I binding to ABCA1 causes adenylate cyclase activation, cAMP production, and subsequent PKA-mediated ABCA1 phosphorylation, which results in increased lipidation of apoA-I (99). There is also evidence of a role for calcium in the modulation of ABCA1 function, with the removal of extracellular calcium or the chelation of intracellular calcium resulting in the attenuation of apoA-I lipidation (100). In addition, protein kinase C (PKC) activation modulates both ABCA1 gene transcription and ABCA1 protein stability. Upon ABCA1 binding, apoA-I initially removes cellular free cholesterol, phosphatidylcholine, and sphingomyelin. The decrease in sphingomyelin in the plasma membrane then triggers phosphatidylcholine phospholipase activity, which catalyzes the hydrolysis of phosphatidylcholine to generate diacylglycerol (DAG). The DAG then activates PKC-α, which phosphorylates ABCA1, ultimately leading to protection of ABCA1 degradation by calpain (101). Interestingly, in response to LDL cholesterol, PKC-ζ activation

induces its binding to the transcription factor specificity protein 1 (Sp1), and Sp1 interaction with LXR and RXR heterodimer and the ABCA1 promoter activates transcriptional transactivation of the ABCA1 gene (102, 103). Along with activating PKA and PKC, the interaction of apoA-I with ABCA1 activates JAK2, resulting in both the enhancement of lipid removal from cells (104) and the activation STAT3, which is anti-inflammatory in cell types such as macrophages (76). Furthermore, Rho family small GT-Pases are also influenced by apoA-I binding to ABCA1. The activation of Cdc42 by apoA-I enhances apoA-I-mediated cholesterol efflux, which may involve the formation of a complex between Cdc42 and ABCA1 (105–107). ApoA-I binding also activates RhoA, which participates in the stabilization of ABCA1 (108).

Structural features of ABCA1 involved in its capacity to alter intracellular signaling and in its modulation by intracellular signaling events have been elucidated. In studies of cAMP production and ABCA1 phosphorylation mediated by the apoA-I/ABCA1 tandem, it was found that mutations of ABCA1 associated with Tangier disease (C1477R, 2203X, and 2145X) cause the attenuation of both responses (99). Mutation of one of the two most likely PKA phosphorylation sites on ABCA1, S2054, yields a transporter that is less capable of exporting cellular cholesterol (109). It has additionally been determined that the C terminus of ABCA1 is required for its interaction with Cdc42, with C-terminal truncation of ABCA1 causing blunted Cdc42-dependent signaling (105). There is also evidence that PDZ-RhoGEF binds to the C-terminal PDZ-binding motif of ABCA1 and prevents the degradation of the transporter by activating RhoA (108). Furthermore, it has been determined that ABCA1 directly binds calmodulin in a calcium-dependent manner. The cytoplasmic loop of ABCA1 contains a calmodulin-binding sequence (residues 1245–1257), and its binding by calmodulin protects ABCA1 from proteolysis by calpain and thereby enhances apoA-I-mediated lipid release (110).

SR-BI

Sometimes independent of and sometimes in partnership with ABCA1 and/or ABCG1, SR-BI is required for the direct actions of apoA-I and HDL on certain cell types. SR-BI is required for HDL modulation of endothelial cell phenotype (56, 60, 64) and for the actions of HDL in EPC (57). SR-BI also mediates the actions of apoA-I and/or HDL in neutrophils (25), adipocytes (111), and pancreatic β cells (36).

The features of SR-BI required for signal initiation have been interrogated. Using SR-BII, which is a splice variant of SR-BI, and chimeric as well as mutant class B scavenger receptors, it has been shown 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 employing a photoactivated derivative of cholesterol, direct binding of plasma membrane cholesterol to the C-terminal transmembrane domain was demonstrated (97). The C-terminal PDZ-interacting domain allows the receptor to bind to the adaptor molecule

PDZK1, which comprises four PDZ domains. Whereas in certain contexts PDZK1 influences the stability of the SR-BI protein, such as in hepatocytes (112), in endothelial cells PDZK1 does not impact SR-BI abundance, and alternatively, it is uniquely required for signal initiation by HDL and the resulting stimulation of eNOS and endothelial cell migration (113).

In recent studies, the functional implications of direct plasma membrane cholesterol binding to the C-terminal transmembrane domain of SR-BI were interrogated. In experiments performed in COS-M6 cells, mutation of a highly conserved C-terminal transmembrane domain glutamine to alanine (SR-BI-Q445A) decreased plasma membrane cholesterol interaction with the receptor by 71% without altering HDL binding or cholesterol uptake or efflux, and it yielded a receptor incapable of HDL-induced signaling. Signaling prompted by cholesterol efflux to methyl-β-cyclodextrin also was prevented, indicating that plasma membrane cholesterol interaction with the receptor enables it to serve as a plasma membrane cholesterol sensor. Using SR-BI-Q445A, it was further demonstrated that plasma membrane cholesterol sensing by SR-BI does not influence SR-BI-mediated RCT to the liver in mice. However, the plasma membrane cholesterol sensing does underlie apolipoprotein B intracellular trafficking in response to postprandial micelles or methyl-β-cyclodextrin in cultured enterocytes, and it is required for HDL activation of eNOS and migration in cultured endothelial cells and HDL-induced angiogenesis in vivo (114). Thus, through interaction with cholesterol, SR-BI serves as a plasma membrane cholesterol sensor, and the resulting intracellular signaling governs processes in both enterocytes and endothelial cells.

UNANSWERED QUESTIONS

Our present understanding of signaling mechanisms, as well as protein or mediator abundance or activity modulated by apoA-I or HDL, is summarized in Tables 1 and 2. The current unanswered questions in this aspect of HDL biology are multiple. First, although the role of HDL in RCT and the capacity of the lipoprotein to directly impact numerous cell types are well recognized, we do not know the relative importance of HDL modulation of global cholesterol homeostasis versus HDL modulation of intracellular signaling. Second, within the latter realm, we do not know to what extent each of the many cellular responses to apoA-I or HDL discussed in this review impacts cardiovascular and/or metabolic health. Third, recognizing that HDL subclasses differ greatly in their many chemical and physical properties and that HDL particles contain a large variety of lipid species and dozens of protein cargos (115), we do not know whether these many characteristics of HDL or its capacity to invoke lipid movement defines the actions of the lipoprotein of importance to health and disease. Finally, from a mechanistic standpoint, although we are improving our understanding of ABCA1, ABCG1, and SR-BI structure-function, we have much more to learn about the initiating

events by which apoA-I binding to these cell-surface proteins causes lipid movement and coupling to intracellular signaling cascades. What we do now appreciate, however, is that the influence of HDL on physiologic and also pathophysiologic processes likely surpasses its classical participation in global cholesterol homeostasis. Further investigations of the bases for and implications of HDL and apoA-I modulation of intracellular signaling will potentially reveal new prophylactic and therapeutic strategies to optimize both cardiovascular and metabolic health.██

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