High-Density Lipoproteins and Endothelial Function

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Abstract—Elevated plasma levels of HDL cholesterol or apolipoprotein A-I, the major protein moiety of HDL particles, are protective against coronary artery disease. HDL particles remove cholesterol from peripheral cells and transfer it to the liver for bile acid synthesis. The interaction between lipoproteins is not mediated through simple contact between 2 phospholipid membranes but involves specific protein-receptor interactions, charged phospholipid-phospholipid contact, and activation of cellular signaling pathways. These lead to regulation of genes or the modification of proteins involved in vasomotor function, platelet activation, thrombosis and thrombolysis, cell adhesion, apoptosis and cell proliferation, and cellular cholesterol homeostasis. (Circulation. 2001;104:1978-1983.)

Key Words: endothelium • lipoproteins • atherosclerosis

Vascular endothelial cells constitute a structurally simple but functionally sophisticated organ that regulates biological processes as diverse as hemostasis, fibrinolysis, inflammation, blood pressure, lipoprotein metabolism, and angiogenesis. Although the endothelium consists of a monolayer of cells, it has an important aggregate surface area and mass. Alterations in one or more of the physiological roles of vascular endothelial cells constitutes endothelial dysfunction. In clinical practice, endothelial function is measured indirectly, by examining the ability of an artery to vasodilate in response to a stimulus that causes NO release in a healthy vascular endothelium. Inhibition of endothelium-dependent relaxation due to the decrease in the bioavailability of NO is the most prominent feature of endothelial dysfunction. An association between atherogenic lipids, such as LDL, and atherosclerosis is strongly supported by epidemiological studies. Several other proteins, including apoA-IV, apoE, clusterin (apoJ), paraoxonase, haptoglobin, α₂-macroglobulin, and lecithin cholesterol acyltransferase, are also associated with HDL and impart significant physiological roles. HDL particles are composed of a monolayer of phospholipids, predominantly phosphatidylcholine (although other phospholipids are present), a relatively small amount of free cholesterol, and a core of cholesteryl esters and a small amount of triglycerides. Nascent HDL particles consist of 2 or 3 molecules of apoA-I arranged radially around a phospholipid bilayer, forming a discoidal structure. This particle is considered the immediate acceptor of membrane cholesterol in the process of reverse cholesterol transport.

In the following sections, we will examine the interactions between HDL and endothelial cells with respect to the various functions of vascular endothelial cells.

Vasodilatation and Vasoconstriction

Nitric Oxide

NO, originally called endothelium-derived relaxing factor, is produced by a constitutive endothelial NO synthase (eNOS) in response to various stimuli, including fluid shear stress and exposure to the neurohumoral factors acetylcholine, bradykinin, serotonin, and substance P. Although short-lived, NO is a potent vasodilator that induces smooth muscle relaxation through the activation of smooth muscle guanylate cyclase. NO also inhibits platelet aggregation and leukocyte adhesion to the endothelium. Inhibition of endothelium-dependent relaxation due to the decrease in the bioavailability of NO is the most prominent feature of endothelial dysfunction. An association between atherogenic lipids, such as LDL, and a decrease in acetylcholine-induced endothelium-dependent relaxation or even a paradoxical vasoconstrictive response is
well documented. High HDL cholesterol in humans, however, is associated with normal endothelium-dependent relaxation in response to acetylcholine.

Incubating isolated rabbit aorta with HDL reverses the decrease in endothelium-dependent relaxation caused by oxidized LDL at physiological HDL concentrations. Exactly how HDL achieves this effect is not entirely clear. Matsuda et al. suggested that HDL acts by preventing the transfer of lysophosphatidylcholine (LPC) from oxidized LDL to endothelial cells. LPC is a major lipid component of oxidized LDL that can mimic many of the atherogenic effects of oxidized LDL, including the decrease in endothelium-dependent vasodilatation. Recently, Uittenbogaard et al. showed that incubation of endothelial cells with oxidized LDL depletes plasma membrane caveolae of cholesterol and translocates eNOS from caveolae to an internal membrane compartment, making eNOS insensitive to stimulation by acetylcholine. HDL opposes this phenomenon by donating cholesterol esters to endothelial cell caveolae and preventing the defective localization of eNOS. eNOS-receptor uncoupling is believed to be important in the early progression of endothelial dysfunction, but in the long term, it is thought that decreases in NO bioavailability are due to an increase in the generation of superoxide anion. Superoxide anion reacts with NO to form peroxynitrate, a less effective vasodilator than NO. HDL is an important antioxidant and may improve the bioavailability of NO by bolstering the antioxidant status of endothelial cells and decreasing the formation of superoxide anion. This concept was recently strengthened by Cominacini et al., who showed that the effect of oxidized LDL is predominantly due to the generation of superoxide anion and not impaired activation of eNOS. The physiological relevance of the effects of HDL on caveola structures and protection against oxidized LDL remains uncertain. Nevertheless, these data suggest that oxidized LDL and HDL have opposing effects on NO bioavailability in endothelial cells.

### Prostaglandins

Prostacyclin (PGI₂) is a prostaglandin produced by endothelial cells that acts on smooth muscle prostacyclin receptors to activate adenylate cyclase and inhibit contraction. Like NO, prostacyclin inhibits platelet aggregation. Prostacyclin increases the synthesis of NO by endothelial cells, and in turn, NO increases the activity of prostacyclin on smooth muscle relaxation. Incubation of HDL with endothelial cells promotes prostacyclin synthesis; HDL also prolongs the half-life of prostacyclin in the blood. As with NO, the mechanism by which HDL increases prostacyclin synthesis is not entirely clear. Pomerantz et al. showed that HDL provides endothelial cells with arachidonic acid in the form of cholesterol arachidonate. The provision of endothelial cells with arachidonate is important, because endothelial cells lack the enzyme required to synthesize arachidonic acid. Spector et al. downplay the contribution of HDL, arguing that arachidonic acid bound to albumin in plasma is more likely to be the source of arachidonic acid for the endothelium in vivo; endothelial cells also use endoperoxides released by platelets for prostaglandin synthesis. Cells prelabeled with radioactive arachidonate and exposed to HDL will generate radiolabeled prostacyclin, suggesting that HDL stimulates the calcium-sensitive phospholipase A₂. Tamagaki et al. showed that IP₃ generation and an increase in intracellular calcium accompany prostacyclin synthesis in endothelial cells incubated with HDL. Furthermore, incubating endothelial cells with HDL and a calcium chelator eliminates the effect of HDL on prostacyclin synthesis, indicating that the effect of HDL is calcium-sensitive and again implicating activation of phospholipase A₂. Cyclooxygenase-2 expression in human umbilical vein endothelial cells is modestly increased by treatment with HDL, and HDL can act synergistically with the inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β to produce a large increase in cyclooxygenase-2 expression. Despite these interactions, the origin of arachidonate as a substrate for prostacyclin synthesis is debated, the data show that HDL mediates production of prostacyclin in vitro.

Thromboxane A₂ (TXA₂) is another prostaglandin produced by endothelial cells, but unlike prostacyclin, TXA₂ is a potent vasoconstrictor and platelet activator. The ratio of prostacyclin to thromboxane is believed to be important for cardiovascular health. HDL can modify this balance. In particular, Oravec et al. found that the HDL₂ subfraction was able to dose-dependently inhibit the formation of TXA₂ in cultured endothelial cells. HDL₃ stimulated TXA₂ formation, but the net effect was a favorable PGI₂:TXA₂ ratio at all but the highest concentrations of HDL used.

### Platelet-Activating Factor

Platelet-activating factor (PAF) is a juxtacrine signaling phospholipid synthesized by activated endothelial cells. PAF stimulates platelet aggregation and smooth muscle contraction via a specific 7-transmembrane-domain PAF receptor. HDL inhibits the synthesis of PAF in activated cultured endothelial cells. This inhibitory activity is independent of the HDL PAF-acetyl hydrolase enzyme and is mediated by a decrease in the activity of cellular acetyl-coenzyme A:lyso-PAF acetyltransferase. Numerous signaling pathways regulate the acetyltransferase, but to date the mechanism used by HDL has not been defined. In human studies, HDL-cholesterol levels correlate inversely with platelet-thrombus formation assessed in a Badimon chamber, strengthening the concept that HDL particles modulate platelet function in vivo.

### Thrombosis and Thrombolysis

#### Primary Hemostasis

NO inhibits platelet aggregation by increasing platelet cGMP generation. HDL restores impaired NO synthesis in endothelial cells that have been exposed to atherogenic lipids. HDL also acts directly on platelets to induce endogenous NO synthesis. Prostacyclin inhibits platelet aggregation by increasing platelet cAMP via a G-coupled protein. As noted before, prostacyclin synthesis in endothelial cells is stimulated by HDL, probably through a combination of substrate provision in the form of cholesterol arachidonate and calcium signaling. Conversely, HDL inhibits the synthesis of the prostaglandin platelet activator TXA₂, further inhibits platelet activation by suppressing a PAF synthetic enzyme in activated endothelial cells. Endothelial cells
express von Willebrand factor (vWF), a platelet ligand essential for platelet adhesion and aggregation in response to vascular injury. Circulating vWF in patients is inversely associated with HDL.20 These various mechanisms may have an overall significance in vivo, because human data indicate an inverse association between HDL and platelet-dependent thrombus formation.18

**Secondary Hemostasis**

Tissue factor pathway inhibitor (TFPI) is secreted by both endothelial cells and the liver and opposes coagulation by acting on the extrinsic pathway. Epidemiological associations between circulating TFPI and HDL in humans are equivocal; one study has shown a negative association between HDL cholesterol and TFPI,21 whereas another showed a positive association between HDL cholesterol and TFPI and apoA-I and TFPI.22

Thrombomodulin is an endothelial membrane protein that binds thrombin, permitting it to activate the anticoagulant serine protease C. Soluble thrombomodulin is often elevated in the plasma of patients with atherosclerosis, but to date no association between HDL and thrombomodulin has been identified. HDL does seem to have an endothelium-independent ability to activate proteins C and S.23

Heparin-like proteoglycans on the endothelial surface further inhibit coagulation by activating antithrombin III. The apoE apolipoprotein moiety on HDL increases the sulfation of these endothelial proteoglycans.24 Other apoE-containing lipoproteins (eg, VLDL) do not behave the same way, for reasons that are not understood.24 Once again, in vivo evidence from humans supports a role for HDL as a thrombin inhibitor, because HDL inversely associates with a marker of activated thrombin.25

**Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1**

Endothelial cells regulate thrombolysis as well as coagulation. The endothelium releases tissue plasminogen activator (tPA) and its inhibitor, plasminogen activator inhibitor-1 (PAI-1), which together control the activity of plasmin, an enzyme that cleaves cross-linked fibrin found in thrombi. In cultured endothelial cells, LDL and glycated LDL decrease the generation of tPA and increase the generation of PAI-1.26 HDL reverses this effect, restoring the normal release of tPA and PAI-1.26 In humans, HDL has not been directly correlated with PAI-1 in the blood,22,27 although one study found an inverse relationship between PAI-1 and the ratio of HDL cholesterol to total cholesterol.27

**Endothelium-Leukocyte Interactions**

Early in the pathogenesis of atherosclerosis, endothelial cells display an increased affinity for leukocytes, which adhere to and migrate through the endothelium, ultimately forming the fatty streak. Increased endothelial expression of the leukocyte adhesion molecules vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and the E- and P-selectins, as well as decreased shear stress and increased leukocyte activation promote leukocyte adhesion to the endothelium. E- and P-selectin are members of the selectin family and are counterreceptors for L-selectin, which is found on all leukocytes. ICAM-1 and VCAM-1 belong to the immunoglobulin superfamily and are counterreceptors for leukocyte integrins. ICAM-1 binds the integrin heterodimer CD11a/CD18 found on all leukocytes and the CD11b/CD18 heterodimer found on monocytes. VCAM-1 binds the integrin heterodimers αβ, αββ, αβ, is found on lymphocytes and monocytes, whereas αβ is found exclusively on lymphocytes. Circulating leukocyte adhesion molecules can be used as molecular markers for atherosclerosis, particularly ICAM-1 and E-selectin, which correlate strongly with coronary artery disease and carotid artery atherosclerosis in humans.28 Oxidized LDL and LPC induce endothelial expression of leukocyte adhesion molecules, as do the inflammatory cytokines TNF-α and IL-1β.29 A number of studies have confirmed that HDL attenuates the cytokine-induced expression of the adhesion molecules VCAM-1, ICAM-1, and E-selectin in cultured endothelial cells.30 Because TNF-α and IL-1β increase the expression of endothelial leukocyte adhesion molecules by activating the transcription factor nuclear factor (NF)-κB, it is hypothesized that HDL inhibits activation of NF-κB. Xia et al31 reported that HDL disrupts the sphingosine kinase signaling cascade that is implicated in cytokine-induced expression of leukocyte adhesion molecules and is mediated by NF-κB activation. Animal experiments show that reconstituted HDL inhibits endothelial VCAM-1 expression and neointima formation in a mouse model of carotid artery injury.32 Reconstituted HDL was used recently to demonstrate inhibition of cytokine-induced E-selectin expression in a porcine model of inflammation.33 Direct comparison from in vitro data to conditions in humans should be made only cautiously, because the concentrations of TNF-α used in experiments often vary anywhere from 100 to 1000 U/mL and thus do not represent normal plasma concentrations of TNF-α. The concentration of TNF-α within the atherosclerotic plaque is not known.

**Endothelial Apoptosis, Proliferation, and Migration**

**Apoptosis**

Apopothisis of endothelial cells has been demonstrated in many cardiovascular diseases, including atherosclerosis. Some atherogenic lipids and inflammatory cytokines are potent apoptotic stimuli for endothelial cells. Defects in the endothelium due to endothelial cell apoptosis would be consistent with the response-to-injury model of atherogenesis; furthermore, an intact endothelium is essential to mask the thrombogenic molecules that lie underneath. HDL exerts a protective effect by interfering with the apoptotic stimuli to which endothelial cells are exposed. Oxidized LDL induces endothelial cell apoptosis in culture by producing a large and sustained increase in intracellular calcium, an increase that HDL opposes.34 The ability of HDL to counteract oxidized LDL involves direct HDL-endothelial cell signaling, because preincubation of oxidized LDL with HDL has no effect on the apoptotic potency of oxidized LDL, and the protective effect of HDL is independent of the HDL antioxidant enzyme paraoxanase.34 Furthermore, HDL does not affect oxidized
LDL uptake by endothelial cells, and cells that are incubated with HDL for >5 hours, washed, and exposed to oxidized LDL continue to be protected. ApoA-I mimics HDL in these respects but is less effective, suggesting that the lipid component of HDL may have some intrinsic antiapoptotic activity. HDL also suppresses the caspases required for apoptosis that are activated by TNF-α, although the mechanism for this has not been explored.

**Proliferation and Migration**

If focal endothelial defects play a part in atherogenesis, then repair of these defects also merits consideration. HDL has long been known to be an important endothelial mitogen. The proliferative effect of HDL on endothelial cells seems to require extracellular calcium and involves changes in intracellular pH. HDL also seems to induce protein kinase C-dependent phosphorylation of a 27-kDa heat-shock protein in proliferating endothelial cells, although the significance of this is not known. Endothelial migration is also important in vessel repair, which HDL promotes through an unknown mechanism, although it is separate from that of basic fibroblast growth factor. Recently, Nofer et al. reported that HDL-derived lysosulfatide and sphingosylphosphorylcholine activate phosphoinositol-specific phospholipase C in fibroblasts and promote cell proliferation. These results have not been reproduced with endothelial cells, yet this suggests that HDL-derived lipids or their metabolites have important physiological actions.

**Signaling**

HDL has thus far been implicated in a variety of endothelial signaling pathways. Several key endothelial behaviors, including proliferation, apoptosis, prostaglandin synthesis, and NO synthesis, are regulated by calcium. HDL can both induce modest increases in intracellular calcium and inhibit large increases in intracellular calcium in cultured endothelial cells. Su et al. showed a biphasic calcium response to HDL, the first phase arising from cellular calcium stores, the second phase representing extracellular calcium. HDL does not require apolipoproteins to increase intracellular calcium in endothelial cells. The isolated lipid fraction of HDL can provoke an increase in endothelial cell calcium concentration. Experiments performed to identify the lipid responsible for the increase in calcium were unsuccessful but rule out lysosphosphatidic acid, LPC, and sphingomyelin as good candidates. Given the recent results derived from fibroblasts, it seems probable that the lipids involved are lysosulfatide and sphingosylphosphorylcholine.

Often closely linked to calcium signaling is phospholipase C-dependent phosphoinositide hydrolysis. Phosphoinositide hydrolysis governs cellular calcium and protein kinase C activation. Phospholipase C activation in endothelial cells by HDL is controversial; in human umbilical vein and aortic endothelial cells, IP3 is generated on exposure to HDL, whereas in bovine aortic endothelial cells, it is not. Protein kinase C activation, which is linked to phosphoinositide hydrolysis and intracellular calcium concentration, has been observed in endothelial cells exposed to HDL, but the significance of this observation has not been explored.

**Cholesterol Balance**

Endothelial cells must cope with exposure to high levels of cholesterol-rich lipoproteins and protect the intima of blood vessels from these same lipoproteins. Despite the high exposure to cholesterol, endothelial cells do not accumulate cholesterol in the same striking manner as atheromatous smooth muscle cells and macrophages. It was recently shown that endothelial cells do not express significant amounts of ABCA1, a protein deeply implicated in cholesterol efflux. As a result, endothelial cells cannot release cholesterol to lipid-free apoA-I. ApoA-I that has been primed with phospholipids, however, can promote cholesterol efflux from endothelial cells. In culture, endothelial cells bind up to 6 times as much HDL as smooth muscle cells. The superior ability to release cholesterol may be an important protective adaptation for endothelial cells. HDL also appears to modulate the permeability of the endothelium to LDL. Large doses of HDL administered to rabbits decreases the appearance of G-proteins are common signaling proteins, and accordingly, Honda et al. showed that HDL calcium signaling in endothelial cells is pertussis toxin-sensitive, indicating G-protein involvement. HDL and LDL appear to influence calcium signaling through the same mechanism, although this is difficult to reconcile with their divergent effects on endothelial function.

HDL has been shown to interfere with the sphingolipid signaling required for TNF-α-dependent expression of endothelial leukocyte adhesion molecules by inhibiting sphingosine kinase. Whether or not interruption of sphingolipid signaling by HDL is important with regard to other endothelial behaviors is not known.

HDL signaling through cholesteryl ester donation is an exciting possibility. The scavenger receptor SR-BI mediates selective cellular uptake of cholesterol from HDL, which has particular significance in steroidogenic tissues. Consistent with this idea is the observation that HDL maintains the appropriate cellular localization of eNOS by replenishing endothelial cellular membranes with cholesteryl esters in an SR-BI-dependent fashion. The possible significance of HDL cholesteryl arachidonate donation for endothelial prostaglandin synthesis also conforms to this idea.

HDL also modulates endothelial pH, which is important for proliferation. HDL does this by affecting the Na+/H+ exchanger and calcium concentrations.
LDL particles in the intima of the aorta. This effect may be due to the competitive inhibition HDL exerts on endothelial LDL uptake.

Conclusions

Although it is now possible to conclude that HDL improves endothelial function (see Figure), fundamental questions regarding the relationship between HDL and endothelial cells remain unanswered. How HDL mediates changes in endothelial phenotype is not thoroughly understood. Investigation of the effects of HDL is strongly warranted, because endothelial dysfunction occurs very early in and is required for atherogenesis. Furthermore, endothelial dysfunction is reversible. Pharmacological modalities, including lipid-lowering agents and the antioxidant probucol, have been shown to improve endothelial function in humans. Knowledge gained from the study of the effects of HDL on endothelial cells may promote the development of new treatments for endothelial dysfunction.

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References


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