High density lipoprotein (HDL) activates endothelial nitric-oxide synthase (eNOS), leading to increased production of the antiatherogenic molecule NO. A variety of stimuli regulate eNOS activity through signaling pathways involving Akt kinase and/or mitogen-activated protein (MAP) kinase. In the present study, we investigated the role of kinase cascades in HDL-induced eNOS stimulation in cultured endothelial cells and COS M6 cells transfected with eNOS and the HDL receptor, scavenger receptor B-I. HDL (10–50 μg/ml, 20 min) caused eNOS phosphorylation at Ser-1179, and dominant negative Akt inhibited both HDL-mediated phosphorylation and activation of the enzyme. Phosphoinositide 3-kinase (PI3 kinase) inhibition or dominant negative PI3 kinase also blocked the phosphorylation and activation of eNOS by HDL. Studies with genistein and PP2 showed that the nonreceptor tyrosine kinase, Src, is an upstream stimulator of the PI3 kinase-Akt pathway in this paradigm. In addition, HDL activated MAP kinase through PI3 kinase, and mitogen-activated protein kinase/extracellular signal-regulated kinase inhibition fully attenuated eNOS stimulation by HDL without affecting Akt or eNOS Ser-1179 phosphorylation. Conversely, dominant negative Akt did not alter HDL-induced MAP kinase activation. These results indicate that HDL stimulates eNOS through common upstream, Src-mediated signaling, which leads to parallel activation of Akt and MAP kinases and their resultant independent modulation of the enzyme.

The risk for cardiovascular disease from atherosclerosis is inversely proportional to serum levels of high density lipoprotein (HDL)1 (1, 2). HDL classically serves to remove cholesterol from peripheral tissues in a process known as reverse cholesterol transport. However, the mechanisms by which HDL is atheroprotective are complex and not fully understood, since circulating levels of HDL and the major HDL apolipoprotein, apolipoprotein A-I, do not regulate reverse cholesterol transport (3). We previously reported that HDL stimulates endothelial nitric-oxide synthase (eNOS) activity in endothelial cells (EC) through apolipoprotein A-I binding to scavenger receptor type I (SR-BI), the high affinity HDL receptor (4). Similarly, HDL enhances endothelium- and NO-dependent relaxation in aortas from wild-type but not SR-BI knock-out mice. Recently, Li et al. (5) also reported that HDL binding to SR-BI activates eNOS. The HDL-induced increase in NO production may be critical to the atheroprotective features of HDL, since diminished bioavailability of endothelium-derived NO has a key role in the early pathogenesis of hypercholesterolemia-induced vascular disease and atherosclerosis (6–8). However, the mechanisms by which HDL activates eNOS are yet to be clarified.

eNOS is one of three isoenzymes that convert L-arginine to L-citrulline plus NO. The activity of eNOS is regulated by complex signal transduction pathways that involve various phosphorylation events and protein-protein interactions. Many stimuli modulate eNOS activity by activating kinases that alter the phosphorylation of the enzyme (9–15). Akt kinase (also known as PKB) activates eNOS by directly phosphorylating the enzyme at Ser-1179 (16–19). Akt itself is phosphorylated and activated by phosphoinositide 3-kinase (PI3 kinase), which in turn is activated by a tyrosine kinase (TK). Both receptor TK and nonreceptor TK are involved in PI3 kinase-Akt-mediated eNOS activation by various agonists (19–22). In contrast to Ser-1179, phosphorylation of Thr-497 of eNOS attenuates enzyme activity (12, 14, 15). eNOS is also modulated by MAP kinases (23, 24), and unlike Akt, the effect of MAP kinases on eNOS activity can be either positive or negative (9, 25–27). The role of kinase cascades in signaling by HDL from SR-BI to eNOS is entirely unknown.

To better understand the basis of HDL action in endothelium, the present investigation was designed to test the hypothesis that HDL activation of eNOS entails the phosphorylation of the enzyme. We also studied the potential roles of specific kinase cascades in HDL-mediated eNOS stimulation. Using pharmacological inhibition or dominant negative mutant forms of selective kinases in EC or COS M6 cells transfected with eNOS and the HDL receptor, SR-BI, we investigated the involvement of tyrosine kinases, PI3 kinase, Akt, and MAP kinases in HDL-mediated eNOS activation. In addition to improving our specific understanding of eNOS modulation, the elucidation of the signaling cascade(s) coupling SR-BI to the enzyme provides important clues about multiple additional potential target of HDL action in EC.

References

1 The abbreviations used are: HDL, high density lipoprotein; eNOS, endothelial nitric-oxide synthase; SR-BI, scavenger receptor B-I; PKB, protein kinase B; PI3 kinase, phosphoinositide 3-kinase; EC, endothelial cell; TK, tyrosine kinase; VEGF, vascular endothelial growth factor; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; CHO, Chinese hamster ovary.
Kinases and HDL Stimulation of eNOS

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Primary ovine endothelial cells were propagated and maintained as described previously (28) in EGM-2 medium from BioWhittaker (Walkervillie, MD). We have shown that SR-BI expression is conserved in these cells up to at least passage 7 (4). For confirmation purposes, selected experiments were also done in human aortic endothelial cells (purchased from BioWhittaker), propagated in EGM-2 and used at passages 4–6, and in bovine aortic endothelial cells, maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% fetal calf serum (Invitrogen). COS M6 cells were transfected with various cDNAs using LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s instruction. The transfected cells were used 48 h after transfection, and 70–80% transfection efficiency was typically achieved. cDNAs for wild-type eNOS, S1179A eNOS, AktAAA, AktMyr, and S65A were prepared as described previously (20). A mutant of eNOS with Ser-1179 converted to alanine (S1179A) or wild-type eNOS for 48 h. The cells were treated with HDL (10 μg/ml) for 20 min, and cell lysates were analyzed by immunoblot using polyclonal anti-phospho-serine 1179 eNOS antibody or monoclonal eNOS antibody. cOS M6 cells were transfected with SR-BI cDNA and either cDNA for wild-type eNOS or S1179A mutant eNOS for 48 h. eNOS activity was then assessed by measuring [3H]-arginine to [3H]-citrulline conversion over 20 min in cells exposed to vehicle alone (basal (B)) or HDL (10 μg/ml). * p < 0.05 versus basal. d, endothelial cells were treated with HDL (30 μg/ml) for 20 min at 37 °C, and cell lysates were analyzed by immunoblot using polyclonal anti-phospho-Thr-495 eNOS, anti-phospho-Ser-1179 eNOS, or monoclonal eNOS antibodies.

**RESULTS**

**HDL Stimulation of eNOS Requires Phosphorylation at Ser-1179**—In order to determine whether HDL stimulation of eNOS requires the phosphorylation of eNOS at Ser-1179, primary ECs were incubated with HDL (10 or 50 μg/ml) for 20 min or with VEGF (100 ng/ml) for 5 min, serving as a positive control. The phosphorylation was detected by Western blotting using anti-phospho-specific eNOS antibody. As shown in Fig. 1a, eNOS phosphorylation was observed with 10 or 50 μg/ml HDL treatment as well as with 100 ng/ml VEGF treatment. Phosphorylation of eNOS by HDL (30 μg/ml) was observed as early as 5 min and reached maximum at 10–20 min (data not shown). Comparable eNOS phosphorylation by HDL was seen in the ovine, human, and bovine EC (data not shown).

We next determined whether the phosphorylation of eNOS at Ser-1179 is required for the activation of eNOS by HDL. Either wild-type eNOS or S1179A mutant eNOS was expressed in COS M6 cells along with SR-BI, and the phosphorylation and activation of eNOS was assessed in response to HDL (10 or 30 μg/ml). No phosphorylation of eNOS by HDL was detected in S1179A-transfected cells, whereas wild-type eNOS phosphorylation was apparent (Fig. 1b). In parallel, HDL did not stimulate eNOS activation in cells transfected with S1179A eNOS, whereas HDL stimulated eNOS activation in wild-type eNOS-expressing cells (Fig. 1c).
Akt/PKB (Akt) antibody. We next examined the effect of PI3 kinase inhibitors on both Akt and eNOS phosphorylation and activation. We investigated the involvement of the PI3 kinase-Akt pathway to ultimately lead to eNOS activation and phosphorylation by HDL. Akt is activated by PI3 kinase through Akt-mediated phosphorylation of the enzyme, we investigated the activation of Akt in the phosphorylation and stimulation of eNOS by HDL. Akt is activated by PI3 kinase through recruitment to the plasma membrane, where Akt becomes phosphorylated at Ser-473 and Ser-308 (31, 35–38). ECs were incubated with HDL (10 or 50 μg/ml) for 20 min. The phosphorylation of Akt at Ser-473 was assessed. In parallel experiments, eNOS activity was measured. Wortmannin inhibited both Akt phosphorylation (Fig. 3a) and eNOS activation (Fig. 3b). In order to examine the involvement of PI3 kinase in eNOS phosphorylation by HDL, the effect of another PI3 kinase inhibitor, LY294002, was assessed. ECs were preincubated with LY294002 (50 μM) for 20 min before HDL (30 μg/ml) stimulation for 0–20 min. As shown in Fig. 3c, LY294002 inhibited HDL-mediated eNOS phosphorylation. To further confirm the involvement of PI3 kinase in eNOS phosphorylation, sham vector or dominant negative PI3 kinase (Sr85) was transfected in COS M6 cells expressing eNOS and SR-BI, and the phosphorylation state of eNOS was assessed. As shown in Fig. 3d, overexpression of dominant negative PI3 kinase inhibited eNOS phosphorylation at Ser-1179. These results suggest that HDL phosphorylates and stimulates eNOS through PI3 kinase-mediated Akt activation.

A Tyrosine Kinase Is Upstream of eNOS Activation by HDL—Tyrosine kinases frequently serve as upstream stimulators of PI3 kinase. The involvement of nonreceptor tyrosine kinase (Src family) has been demonstrated for eNOS stimulation by a variety of factors (21, 22). We determined the effect of the tyrosine kinase inhibitor, genistein, on eNOS activation and phosphorylation by HDL. Primary cultured endothelial cells were preincubated with or without Genistein (50 μM) for 20 min before the addition of HDL (30 μg/ml) for 0–20 min. Phosphorylation states and activation of eNOS were assessed. As shown in Fig. 4, a and b, Genistein abrogated both eNOS phosphorylation and activation. We next examined the involvement of the Src kinase family. Endothelial cells were incubated with or without the Src kinase-specific inhibitor, PP2 (0.1 μM), before HDL stimulation (10 μg/ml, 20 min). PP2 inhibited both eNOS activation and phosphorylation by HDL (Fig. 4, c and d). These results suggest that HDL stimulates tyrosine kinases that are most likely Src family kinases, which in turn activate the PI3 kinase-Akt pathway to ultimately lead to eNOS phosphorylation.

**HDL Activation of MAP Kinase**—The role of MAP kinases in HDL stimulation of eNOS is not known. As such, we studied the effect of HDL on MAP kinase activation in primary ECs.
Cells were stimulated with HDL (10 or 50 μg/ml) for 20 min, using fetal calf serum (10%, 5 min) or VEGF (100 ng/ml, 5 min) as a positive control. The activation state of MAP kinase was assessed by phospho-MAP kinase-specific antibody. As shown in Fig. 5a, HDL stimulated MAP kinase phosphorylation. The phosphorylation was detected as early as 2 min and reached maximal phosphorylation at 30 min (Fig. 5b). The activation of MAP kinase by HDL was suppressed by the MEK inhibitor PD98059 (Fig. 5c). Both PI3 kinase inhibition (wortmannin (Wort)) (Fig. 5d) and Src kinase inhibition (PP2) (Fig. 5e) also

Fig. 3. PI3 kinase is involved in HDL-induced, Akt-mediated eNOS phosphorylation and activation. a, endothelial cells were pretreated with vehicle alone, or the PI3 kinase inhibitor wortmannin (Wort; 50 μM) for 30 min. The cells were then incubated with HDL (30 μg/ml) for 20 min, and cell lysates were analyzed by immunoblot using anti-phospho-Ser-473 Akt/PKB (pAkt) polyclonal antibody or anti-Akt polyclonal antibody. b, after pretreatment with vehicle alone or wortmannin (HDL + Wort), eNOS activity was assessed by measuring [3H]-arginine to [3H]-citrulline conversion over 20 min in cells exposed to vehicle (basal (B)), HDL (10 μg/ml), or HDL plus wortmannin (50 μM). *, p < 0.05 versus basal; †, p < 0.05 versus HDL alone. c, endothelial cells were pretreated with vehicle alone or the PI3 kinase inhibitor LY294002 (50 μM) for 30 min. The cells were then treated with HDL (30 μg/ml) for 0, 10, or 20 min at 37 °C. Cell lysates were analyzed by immunoblot using anti-phospho-Ser-1179 eNOS polyclonal antibody (peNOS) or anti-eNOS monoclonal antibody (eNOS). d, COS M6 cells were transfected with SR-BI and eNOS cDNAs and with cDNA for either vector (sham) or dominant negative PI3 kinase (SrΔp85) for 48 h. The cells were then treated with HDL (30 μg/ml) for 20 min, and cell lysates were analyzed by immunoblot using anti-phospho-Ser-1179 eNOS polyclonal antibody (peNOS), anti-eNOS monoclonal antibody (eNOS), or the anti-p85 subunit of PI3 kinase polyclonal antibody.

Fig. 4. Tyrosine kinase is involved in eNOS phosphorylation and stimulation by HDL. a, endothelial cells were pretreated with vehicle or genistein (50 μM) for 30 min and then stimulated with HDL (30 μg/ml) for 0, 10, or 20 min. Cell lysates were analyzed by immunoblot using anti-phospho-Ser-1179 eNOS polyclonal antibody (peNOS) or anti-eNOS monoclonal antibody (eNOS). b, after pretreatment with vehicle or genistein, eNOS activity was assessed by measuring [3H]-arginine to [3H]-citrulline conversion over 20 min in cells exposed to vehicle (basal (B)), HDL (10 μg/ml), or HDL plus genistein (50 μM). *, p < 0.05 versus basal; †, p < 0.05 versus HDL alone. c, endothelial cells were pretreated with PP2 (0.1 μM) for 30 min and exposed to vehicle (B) or HDL (10 μg/ml) for 20 min. eNOS activity was assessed as described for b. *, p < 0.05 versus basal; †, p < 0.05 versus HDL alone. d, endothelial cells were pretreated with PP2 (0.1 μM) for 30 min and exposed to vehicle or HDL (30 μg/ml) for 20 min. Cell lysates were analyzed by immunoblot using anti-phospho-eNOS polyclonal antibody (peNOS) or anti-eNOS monoclonal antibody (eNOS).
attenuated MAP kinase activation by HDL, indicating that PI3 kinase and Src kinase are the upstream effectors of MAP kinase activation by HDL. To determine whether MAP kinase phosphorylation is required for eNOS activation by HDL, EC were pretreated with MEK inhibitor (PD98059), and eNOS activation was assessed. As shown in Fig. 5f, PD98059 (50 μM) completely abrogated eNOS activation by HDL. Comparable MAP kinase activation by HDL was observed in the ovine, human, and bovine EC (data not shown).

To determine whether MAP kinase activates eNOS through the phosphorylation of the enzyme at Ser-1179, HDL-stimulated Akt phosphorylation at Ser-473, one of the major phosphorylation sites of Akt in the pathway leading to eNOS activation through a different mechanism.

To further assess possible cross-talk between Akt and MAP kinase signaling pathways, we determined whether the MAP kinase cascade activates Akt. EC were treated with the MEK inhibitor, PD98059, and the phosphorylation of Akt was assessed. As shown in Fig. 6b, MEK inhibition had no effect on Akt phosphorylation by HDL, suggesting that MAP kinase is not an upstream effector of Akt. We also determined the effect of an Akt dominant negative mutant on MAP kinase phosphorylation by HDL. As shown in Fig. 6c, dominant negative Akt had no effect on HDL stimulation of MAP kinase, but the phosphorylation of eNOS was fully prevented. This suggests that Akt activation is not required for MAP kinase phosphorylation induced by HDL.

DISCUSSION

In the present study, we have demonstrated that HDL stimulation of eNOS occurs through two kinase pathways (Fig. 7). By binding to SR-BI, HDL causes eNOS phosphorylation at Ser-1179 via TK-PI3 kinase-mediated activation of Akt kinase. However, concomitant TK-PI3 kinase-mediated stimulation of MAP kinase is necessary in order to enhance eNOS enzymatic activity in response to the lipoprotein.

The phosphorylation of eNOS regulates the activation of the enzyme by various stimuli including VEGF, estrogen, and shear stress (16–19). We have found that HDL also causes the phosphorylation of eNOS at Ser-1179 and that the phosphorylation is required for the activation of enzymatic activity (Fig. 1, a–c). In contrast to Ser-1179, HDL had no effect on the phosphorylation state of Thr-497 (Fig. 1d), suggesting that the lipoprotein does not regulate eNOS activation through the dephosphorylation of that residue. We also identified Akt as the kinase responsible for HDL-induced eNOS phosphorylation at Ser-1179 (Fig. 2, a–d). HDL stimulated Akt phosphorylation at Ser-473, one of the major phosphorylation sites of Akt in the regulatory domain that is often targeted by PI3 kinase (Fig. 2, a and b). The kinase-inactive mutant of Akt (AktAAA) efficiently inhibited HDL-induced eNOS phosphorylation. Furthermore, we determined whether PI3 kinase is an upstream regulator of Akt in the pathway leading to eNOS activation (Fig. 3, a–d). PI3 kinase activates Akt by recruiting the latter to the plasma membrane, which allows the phosphorylation of Akt at two key regulatory sites (Thr-308 and Ser-403), and Akt was recruited to plasma membrane by HDL stimulation (Fig.
The inhibition of PI3 kinase by wortmannin resulted in decreased Akt phosphorylation at Ser-473 (Fig. 3a). In addition, the selective inhibitor of PI3 kinase LY294002 or dominant negative PI3 kinase also led to decreased eNOS phosphorylation and activation by HDL (Fig. 3, b–d). These cumulative results indicate that PI3 kinase stimulation of Akt leading to eNOS phosphorylation at Ser-1179 is critically involved in the activation of the enzyme by HDL (Fig. 7).

Additional proximal signaling events have been elucidated. We have demonstrated that a protein TK, most likely an Src family kinase, is a further upstream stimulator of the PI3 kinase/Akt pathway (Fig. 4, a–d). Typical PI3 kinase has regulatory subunits with two Src homology 2 domains that allow the enzyme to be activated by phosphotyrosine residues of a TK (39, 40). We speculate that HDL binding to SR-BI directly or indirectly causes tyrosine phosphorylation of Src kinase. Both the C-terminal and N-terminal domains of SR-BI are facing the cytoplasm and thus available for interaction with other proteins. At present, there is no evidence that SR-BI binds to a receptor TK or a nonreceptor TK. Further, it is not known whether HDL causes the phosphorylation of a tyrosine residue within the C-terminal cytoplasmic domain of SR-BI, which could potentially bind to Src homology 2-containing adaptor molecules. Detailed studies of possible SR-BI-tyrosine kinase interactions are now warranted.

In addition to modulation by PI3 kinase/Akt, MAP kinases are also known to play a role in eNOS regulation by certain agonists (9, 25–27). In the present study, we have shown that HDL stimulates MAP kinase phosphorylation (Fig. 5, a–c). Typical PI3 kinase has regulatory subunits with two Src homology 2 domains that allow the enzyme to be activated by phosphotyrosine residues of a TK (39, 40). We speculate that HDL binding to SR-BI directly or indirectly causes tyrosine phosphorylation of Src kinase. Both the C-terminal and N-terminal domains of SR-BI are facing the cytoplasm and thus available for interaction with other proteins. At present, there is no evidence that SR-BI binds to a receptor TK or a nonreceptor TK. Further, it is not known whether HDL causes the phosphorylation of a tyrosine residue (tyrosine 489) within the C-terminal cytoplasmic domain of SR-BI, which could potentially bind to Src homology 2-containing adaptor molecules. Detailed studies of possible SR-BI-tyrosine kinase interactions are now warranted.

Moreover, dominant negative Akt had no effect on MAP kinase activation by HDL (Fig. 6c). Thus, there is no cross-talk between the Akt kinase and MAP kinase pathways, and the activation of both pathways is necessary for enhanced enzymatic activity in response to HDL (Fig. 7).

In addition to modulation by PI3 kinase-Akt, MAP kinases are also known to play a role in eNOS regulation by certain agonists (9, 25–27). In the present study, we have shown that HDL stimulates MAP kinase phosphorylation (Fig. 5, d and e). It has been previously observed that HDL stimulates MAP kinase in EC as well as other cell types (41–43). However, the mechanisms mediating that process were not known prior to the current studies. Furthermore, the activation of MAP kinase is absolutely required for HDL-induced stimulation of eNOS (Fig. 5f). However, MAP kinase activation does not play a role in HDL-induced Akt phosphorylation (Fig. 6b) or in eNOS phosphorylation (Fig. 6e). Moreover, dominant negative Akt had no effect on MAP kinase activation by HDL (Fig. 6e). Thus, there is no cross-talk between the Akt kinase and MAP kinase pathways, and the activation of both pathways is necessary for enhanced enzymatic activity in response to HDL (Fig. 7). Further experiments will be needed to elucidate the mechanism(s) by which MAP kinase contributes to eNOS stimulation by HDL, including the potential modulation of intracellular Ca2+ homeostasis.
Recently, Li et al. (5) reported that HDL stimulates eNOS in a ceramide-dependent manner. C2-ceramide stimulates MAP kinase via tyrosine kinase and PI3 kinase-mediated mechanisms in cultured airway smooth muscle cells (44, 45). It is possible that the HDL stimulation of MAP kinase observed in the present work occurred through ceramide production. Li et al. (5) also showed that HDL does not induce Akt kinase activation in CHO cells expressing eNOS and SR-BI. However, their studies were limited to assessments of Akt phosphorylation in the transfected CHO cells. Discrepancies between the observations made in the two studies may be related to the use of different cell paradigms. In the present work, we used ECs for endogenous Akt phosphorylation and COS M6 cells for cell transfection in which dominant negative mutants were employed to show that Akt is responsible for eNOS phosphorylation and activation (Fig. 2). We also used a combination of PI3 kinase inhibitors (Fig. 3) and dominant negative mutants (Figs. 2 and 3) to confirm the involvement of the PI3 kinase/Akt pathway in HDL-induced eNOS phosphorylation and activation. The eNOS phosphorylation observed in the ovine EC was also confirmed in the human and bovine endothelium. As such, multiple approaches have been employed in the current studies to implicate a key role for Akt kinase and phosphorylation in eNOS activation by HDL.

SR-BI is the high affinity HDL receptor, and HDL binding to SR-BI is required for HDL-mediated cholesterol flux (46, 47). We showed previously that HDL binding to SR-BI is also required for eNOS activation by HDL (4). However, the initiating event that occurs upon HDL binding to SR-BI to cause the proximal processes in signal transduction by the lipoprotein is not known. We speculate that SR-BI mediates the effect of HDL by two possible mechanisms. First, alterations in membrane cholesterol pools may be involved. SR-BI mediates changes in the cholesterol content of the plasma membrane, and SR-BI and eNOS both reside in cholesterol-rich microdomains known as caveolae, which are most likely a subset of lipid rafts, which contain various signaling molecules including MAP-kinase. It has been demonstrated that cholesterol alterations induced by β-cycloexetrin activate MAP-kinases (48). Along with potential cholesterol-related mechanisms, there may be involvement of SR-BI C-terminal binding protein(s). Using antibody blockade in isolated EC plasma membranes, we previously showed that the C-terminal domain of SR-BI plays a role in HDL-mediated eNOS stimulation (4). It is possible that a protein binding to the C terminus of SR-BI mediates signal transduction from SR-BI to a downstream effector such as a G protein and/or Src kinase. A PDZ-containing protein, PDZK1 (also known as CLAMP, Diphor-1, CAP70, or NaPi-Cap1) has been shown to bind to the C-terminal domain of SR-BI and to be involved in cholesterol regulation (49). Now knowing that a Src kinase is critically involved in the proximal signalizing events induced by HDL binding to SR-BI, in depth experimentation focused on the possible roles of both cholesterol regulation and SR-BI adaptor proteins in the upstream process can be pursued.

The intricate regulation of kinase cascades by HDL shown in this study may help explain the impact of HDL on various other functions in EC. For example, HDL stimulates the migration and proliferation of EC (50–52). Since Akt is known to have a role in apoptosis, and MAP kinase is involved in the proliferation and migration of EC during reendothelialization after injury to the arterial wall (53, 54), it is possible that the modulation of these pathways by HDL may be critical to the regulation of EC turnover and movement. As such, our finding that HDL is a potent stimulus of various kinases in EC enhances both our specific understanding of the capacity of the lipoprotein to modulate NO production and our overall knowledge of other mechanisms by which HDL may be atheroprotective.

Acknowledgments—We thank Helen Hobbs (McDermott Center for Human Growth and Development and Departments of Molecular Genetics and Biochemistry) for providing HDL and for insightful discussions and Divya Seetharam for valuable help with experiments of bovine aortic endothelial cells. We are indebted to Marilyn Dixon for preparing this manuscript.
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High Density Lipoprotein-induced Endothelial Nitric-oxide Synthase Activation Is Mediated by Akt and MAP Kinases

Chieko Mineo, Ivan S. Yuhanna, Michael J. Quon and Philip W. Shaul

J. Biol. Chem. 2003, 278:9142-9149.
doi: 10.1074/jbc.M211394200 originally published online January 2, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M211394200

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