Apolipoprotein E Containing High Density Lipoprotein Stimulates Endothelial Production of Heparan Sulfate Rich in Biologically Active Heparin-like Domains

A POTENTIAL MECHANISM FOR THE ANTI-ATHEROGENIC ACTIONS OF VASCULAR APOLIPOPROTEIN E

(Received for publication, June 19, 1998, and in revised form, December 8, 1998)

Latha Paka, Yuko Kako, Joseph C. Obunike, and Sivaram Pillarsetti†

From the Division of Preventive Medicine and Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032

Reduction and heparan sulfate (HS) proteoglycans (PG) have been observed in both inflammation and atherosclerosis. Methods to increase endogenous heparan and heparan sulfate are not known. We found that incubation of endothelial cells with 500–1,000 μg/ml high density lipoprotein (HDL) increased 35SO4 incorporation into PG by 1.5–2.5-fold. A major portion of this increase was in HS and was the result of increased synthesis. Total PG core proteins were not altered by HDL; however, the ratio of 35SO4 to [3H]glucosamine was increased by HDL, suggesting increased sulfation of glycosaminoglycans. In addition, HDL increased the amount of highly sulfated heparin-like HS in the subendothelial matrix. HS from HDL-treated cells bound 40 ± 5% more 125I-antithrombin III (requires 3-O sulfated HS) and 49 ± 3% fewer monocytes. Moreover, the HS isolated from HDL-treated cells inhibited smooth muscle cell proliferation (by 63 ± 5%) better than control HS (56 ± 6%) and heparin (42 ± 6%). HDL isolated from apolipoprotein E (apoE)-null mice did not stimulate HS production unless apoE was added. ApoE also stimulated HS production in the absence of HDL. ApoE did not increase 35SO4 incorporation in macrophages and fibroblasts, suggesting that this is an endothelial cell-specific process. Receptor-associated protein inhibited apoE-mediated stimulation of HS only at higher (20 μg/ml) doses, suggesting the involvement of a receptor-associated protein-sensitive pathway in mediating apoE actions. In summary, our data identify a novel mechanism by which apoE and apoE-containing HDL can be antiatherogenic. Identification of specific apoE peptides that stimulate endothelial heparin/HS production may have important therapeutic applications.

Proteoglycans (PG),1 important constituents of vascular cell membranes and extracellular matrix (1, 2), consist of a core protein to which long chains of negatively charged polysaccharides termed glycosaminoglycans (GAG) are attached. The three major PG classes in the vessel wall are heparan sulfate (HS), chondroitin sulfate, and dermatan sulfate. HSPG play an important role in the regulation of various vascular functions. They bind and promote lipoprotein lipase activity, the key enzyme in the hydrolysis of triglyceride-rich lipoproteins (3). Basic fibroblast growth factor, a potent mitogen and angiogenic factor, requires the presence of cell surface HSPG or exogenous heparin to bind to its high affinity cell signaling receptor (4). In addition, HSPG potentiates the thrombin-inhibiting actions of antithrombin (5).

A reduction in arterial HS and heparin has been observed under conditions of inflammation and atherosclerosis as well as with increased age (6–14). The age-dependent decrease in HS is more pronounced in atherosclerotic tissues than in normal tissues (10, 11). An inverse correlation between the amount of cholesterol in the lesion and the concentration of HS was observed in human aortas. More importantly, this negative correlation was observed in both normal and atherosclerotic vessels. 4–5-fold more cholesterol was found in vessels that have 50% less HS.

The negative relationship between HS and atherosclerosis is not surprising because arterial HS is known to inhibit smooth muscle cell (SMC) proliferation and promote antithrombin activity (5, 15–17). In addition, our recent studies show that HS masks subendothelial proteins such as fibronectin and prevents lipoproteins such as lipoprotein(a) and monocytes from associating with the matrix (18, 19). Thus, an increase in vascular HSPG could be athero-protective. Although the decrease of HSPG in atherosclerosis has been known for several years, it is not clear how this occurs. In vitro, a reduction in subendothelial HSPG was observed when endothelial cells were exposed to moderately oxidized LDL or lyssolecithin, a product of lipoprotein oxidation. This reduction in subendothelial HSPG was found to be caused by secretion, by endothelial cells, of a HSPG-degrading heparanase activity (18, 19). During these studies an initial observation was made that HDL blocked the oxidized LDL- and lyssolecithin-mediated decreases in HSPG (19). We now show that HDL, more specifically the apoE component of HDL, stimulates endothelial HS synthesis.

MATERIALS AND METHODS

[35S]Sulfate aqueous solutions were from Amersham Pharmacia Biotech. Heparinase I and heparitinase (heparinase III) and chondroitin ABC lyase were purchased from Seikagaku America Inc. (Bethesda, Maryland). CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; BSA, bovine serum albumin; WT, wild type.
MD). Receptor-associated protein (RAP) was kindly provided by Dr. D. K. Strickland (Bethesda, MD). For experiments with apoE either a recombinant apoE3 (kindly provided by Dr. M. Al-Heidari of Columbia University (20)) or a commercially obtained apoE3 (Calbiochem) was used.

Lipoproteins—VLDL (d < 1.006), LDL (d < 1.063), and HDL (d = 1.0–1.21) were isolated from fresh human plasma in the presence of EDTA by sequential ultracentrifugation and dialyzed against phosphate-buffered saline containing 0.5 mM EDTA (20). For mouse HDL, blood was drawn by retroorbital bleeding from wild type (C57BL/6J), apoE-null (genetic background C57BL/6J), and apoA-I-null mice; HDL was isolated as above.

Cells—Bovine aortic endothelial cells were isolated and cultured as described (21). The cells (5–15 passages) were grown in minimal essential medium (MEM) containing 10% fetal bovine serum (Life Technologies). The subendothelial matrix was prepared as described previously (18) by incubation for 5 min in a solution containing 20 mM NH4OH and 0.1% Triton X-100 at room temperature.

Metabolic Labeling—Endothelial PG were radiolabeled with either [35S]sulfate or [3H]glucosamine for the indicated time periods. Cell-associated PG were assessed by removing the cells with NH4OH/Triton X-100 as described above. Subendothelial matrix PG were extracted by incubation with 6 M guanidine hydrochloride for 4 h. Alternatively, the subendothelial matrix was incubated with either heparanase/heparitinase or chondroitinase ABC, and released radioactivity was measured to assess HS and chondroitin sulfate/dermatan sulfate PG. Total endothelial cell proteins were labeled by incubating the cells with [3H]leucine for 16 h at 37 °C. Protein synthesis was assessed either by extraction of cells with 0.1 N NaOH and 0.1% SDS for 1 h or by precipitation with 10% trichloroacetic acid.

To study the effects of HDL and other lipoproteins, endothelial cells were incubated in culture medium containing 35SO4 and the indicated lipoprotein or apoE (free or in dimyristoylphosphatidylcholine (DMPC) vesicles; see Ref. 56) for 16 h. Cell and matrix concentrations of the lipoprotein or apoE (free or in dimyristoylphosphatidylcholine (DMPC) vesicles; see Ref. 56) were determined in isolated GAG. To prepare GAG chains, aliquots of dialyzed supernatants were counted, and radioactivity was expressed as disintegrations per minute (dpm).

ApoE HDL Stimulates Endothelial Heparan Sulfate

RESULTS

HDL Stimulates Endothelial PG Production—To determine the effects of HDL on PG, confluent monolayers of endothelial cells were incubated with HDL for 16 h. A dose curve of HDL versus 35SO4 incorporation in cells, matrix (Fig. 1A), and medium (Fig. 1B) is shown. 35SO4 incorporation in all three pools, secreted, cellular, and matrix, increased by 50–100%. An increase of 43% with 250 μg of HDL protein and an increase of 83% with 500 μg of HDL protein was observed in matrix. HDL also increased [3H]leucine incorporation into proteins by 15–20% (not shown). However, the increase in PG sulfate shown in Fig. 1 was found after normalizing for protein. These data suggest that HDL stimulated sulfated incorporation into PG. It should be noted that these changes were found using levels of HDL which are within the normal plasma concentrations of HDL protein (150–180 mg/dl fasting plasma (24)).

HDL Increases Endothelial Cell PG Synthesis—To determine whether the HDL-mediated increase in sulfate incorporation was caused by increased synthesis, cells were labeled with 35SO4 for different time periods in the presence of HDL (1,000 μg/ml). HDL increased sulfate incorporation by 25% in 2 h and by 37% in 6 h in cells (Fig. 2A) and matrix (Fig. 2B). When endothelial PG first labeled with [35S]SO4 and chased in cold medium for different time periods in the presence or absence of HDL, HDL did not alter the turnover rates of PG (not shown). These data suggest that HDL-mediated increases in [35S]SO4 incorporation were primarily the result of increased synthesis.

HDL Did Not Alter PG Core Proteins but Increased Sulfitation of GAG—To determine if increased [35S]SO4 incorporation was caused by increased PG core proteins or increased GAG synthesis, cells were labeled either with [3H]leucine (to label pro-
teins) or with \[^{3}H\]glucosamine (to label GAG). To differentiate \[^{3}H\]leucine incorporation into PG core proteins versus other cellular proteins, DEAE-cellulose chromatography was performed. In different experiments we showed that under the conditions, 90% of bound \(^{35}S\)O\(_4\) radioactivity was eluted at salt concentrations of 0.25 M NaCl. When equal amounts of cellular proteins were loaded on DEAE, the amount of radioactive eluted at 0.5 M NaCl was not different (Fig. 3, 2,992 cpm in control cells versus 3,079 cpm in HDL-treated cells). Although this suggests that total core proteins were not altered, whether HDL altered core proteins of specific PG (such as perlecan and syndecans, which may not reflect in total core proteins) remains to be determined.

HDL increased endothelial GAG (\[^{3}H\]glucosamine incorporation into PG; Fig. 4A), by 34% in cells and by 41% in matrix. Thus, the increase in \(^{35}S\)O\(_4\) incorporation into PG was, in part, the result of increased GAG synthesis. Endothelial cells primarily synthesize HS; consistent with this, >75% of total GAG was resistant to chondroitin ABC lyase (which removes chondroitin and dermatan sulfates) treatment (not shown). Enrichment in GAG could be caused by either an increased number of GAG or an increased chain length. Sizing analysis of isolated GAG by Sepharose 6B gel filtration chromatography did not show changes in the chain length of GAG isolated from control and HDL-treated cells (not shown). We then compared the increases in sulfation and GAG (expressed as sulfate cpm/glucosamine cpm) (Fig. 4B). Although HDL increased glucosamine (Fig. 4A), the GAG were relatively more sulfated in HDL-treated cells as indicated by an increase in the sulfate:glucosamine ratio. A 2.5-fold increase in the sulfation of cellular GAG and an increase of 1.5-fold in the sulfation of matrix GAG was observed.

**Anti-atherogenic and Anti-thrombogenic Properties of HS from HDL-treated Endothelial Cells**—Several anti-atherogenic actions of HS require specific sulfate groups. Studies from this laboratory showed that subendothelial HS inhibition of monocyte binding require sulfation (18). Acceleration of antithrombin-induced proteinase inhibition by HS requires a pentasaccharide with 3-O sulfate on the internal glucosamine (2). The anti-proliferative activity of arterial HS appears to require sequences rich in 2-O sulfated uronic acid (25). Similarly, HS that contains 2-O iduronic acid has a high affinity for lipoprotein lipase (26). These sequences are generally localized to heparin-like domains in HS (resistance to heparitinase). In our experiments we found a 1.7-fold increase in these heparitinase-resistant but nitrous acid-sensitive heparin-like sequences in matrix (cpm/well in a six-well plate, 10,532 ± 675 in control versus 17,986 ± 1,289 in HDL-treated cells).

To determine the consequences of increased matrix HS, ma-
with buffers containing 0.25M NaCl and 0.5 M NaCl. The majority of extracted and subjected to DEAE-cellulose chromatography and eluted with buffers containing 0.25 M NaCl and 0.5 M NaCl. The majority of labeled proteins eluted at 0.25 M NaCl. Total radioactivities eluting at 0.5 M NaCl (containing mostly PG) were similar in control and HDL-treated endothelial cells.

trix was prepared from control and HDL-treated endothelial cells and incubated either with [3H]leucine-labeled monocytes or 125I-labeled antithrombin III. The number of monocytes binding to the HDL matrix was decreased by approximately 49%, suggesting that increased HSPG inhibited monocyte interactions with subendothelial matrix (Fig. 5). In contrast, antithrombin III binding to matrix was increased by 43% in HDL-treated cells compared with control matrix. These data suggest an increase in specific HS (containing 3-O sulfates).

We next tested the ability of the matrix HS to inhibit SMC proliferation, a key event in the development of atherosclerosis. SMC were cultured in the presence or absence of matrix HS isolated from control and HDL-treated endothelial cells. Cell proliferation was assessed either by counting the number of cells (Fig. 6) or by assessing [3H]thymidine incorporation. Endothelial HS was normalized for glucosamine concentration before adding to SMC. Incubation of SMC with HS isolated from chlorate (an inhibitor of sulfation)-treated endothelial cells did not significantly inhibit SMC growth, suggesting the requirement for sulfated HS. Heparin, a known inhibitor of SMC proliferation, at 50 units/ml inhibited SMC proliferation by 42 ± 6%. HS prepared from HDL-treated endothelial cells showed most inhibition (82 ± 5%), better than heparin and control HS (56 ± 6%). These data suggest that HDL treatment increased antiproliferative HS in endothelial cells.

**HDL Effects Require ApoE**—We next tested whether HDL effects on PG sulfation require apoA-I and/or apoE. HDL was isolated from wild type (WT), apoA-I-null, and apoE-null mice and tested for its ability to stimulate sulfation. Equal amounts of HDL-protein (100 µg/ml) were used. HDL isolated from control and apoA-I-null mice increased 35SO4 incorporation into cellular PG. HDL from apoE-null mice, in contrast, failed to stimulate endothelial PG sulfation (Fig. 7A). Similar results were obtained when apoE HDL was removed from human HDL by heparin-Sepharose chromatography; the E-deficient HDL did not increase PG sulfation (data not shown). To determine further if this lack of stimulation was caused by the absence of apoE, apoE was added to apoE-null HDL (Fig. 7A). The addition of 5 µg of apoE restored the ability of E-null HDL to stimulate PG sulfation to mouse WT HDL levels. At 10 µg of apoE, E-null HDL increased 35SO4 incorporation by 2-fold. These data show that apoE is required to stimulate PG sulfation.

We further examined the effects of apoE in emulsions and in lipid free form. ApoE was able to stimulate endothelial HS production in both free form and DMPC emulsions (Fig. 7B). These data suggest that apoE actions do not require other HDL components. Surprisingly, however, VLDL failed to stimulate HS production despite containing similar or greater amounts of apoE (determined by SDS-polyacrylamide gel electrophoretic analysis; not shown).

We next tested whether apoE would stimulate 35SO4 incorporation in other cells. Unlike in endothelial cells, incubation of J774 macrophages (which do not synthesize apoE) or human skin fibroblasts with apoE (10 µg/ml) did not alter PG production (Table I).

**Endothelial Cell Surface Molecules Involved in ApoE Actions**—We next determined the role of lipoprotein receptors in mediating apoE actions. Competition with lipoproteins and receptor antagonists were used (Fig. 8). LDL or VLDL neither stimulated HS production (closed bars) nor inhibited HDL-mediated stimulation (open bars). Similar results were obtained with LDL receptor antibody. The 39-kDa RAP at con-
centrations that others have used to inhibit LDL receptor-related protein (5–10 m̄g) did not inhibit an HDL-mediated increase in 35SO4 incorporation. However, at a higher dose (20 m̄g/ml) RAP inhibited apoE-mediated HS production by 60%.

The requirement for higher doses of RAP could be caused by the longer incubation times (16 h) required for HS stimulation in the current experiments. Nevertheless, these data suggest that RAP-sensitive pathways are involved in apoE function. In different experiments heparin increased 35SO4 incorporation by 25–30%. HDL effects, however, were less pronounced in the presence of heparin. These data indicate that cell surface HSPG may partly contribute to apoE actions.

**PG in ApoE-null Mice**—To test whether apoE would affect PG sulfation in vivo, 35SO4 incorporation was determined in WT and apoE-null mice. 4-week-old mice were injected with 35SO4, and incorporation into PG was determined after 4 ha as described under “Materials and Methods.” Values represent the mean ± S.D.

**FIG. 5.** Effect of increased matrix HSPG on monocyte and antithrombin binding. THP-1 monocytes were labeled with [3H]leucine (100 μCi/1 × 10⁷ cells) for 2 h at 37 °C. Label was removed, and the cells were washed three times with MEM-BSA and suspended in MEM-BSA. Suspended cells were added to matrix prepared from control or HDL-treated endothelial cells in 24-well plates (2–4 × 10⁵ cells/well) and incubated for 1 h at 37 °C. Unbound monocytes were removed by washing four times with MEM-BSA, and bound radioactivity was extracted by incubation in 0.1 N NaOH and 0.1% SDS for 1 h. Antithrombin was iodinated using the lactoperoxidase/glucose oxidase method and purified by heparin-agarose chromatography. 5 μg of iodinated protein was incubated with matrix prepared from control and HDL-treated cells for 2 h at 37 °C. Unbound protein was removed, and bound radioactivity was determined.

**FIG. 6.** HS isolated from HDL-treated endothelial cells is a potent inhibitor of SMC proliferation. SMC were plated at low density (8 × 10⁴/well) and cultured for 3 days in medium alone (None) or media containing 50 units/ml heparin, matrix HS isolated from 25 μM chlorate-treated, control, and HDL-treated endothelial cells. On the 4th day, the cell number was determined. The percentage of SMC growth inhibition was calculated as described under “Materials and Methods.”

**TABLE I**

Effect of apoE on 35SO4 incorporation in different cell types

Aortic endothelial cells or J774 macrophages or fibroblasts were grown to confluence and incubated in growth medium containing 25 μCi/ml 35SO4 with (10 μg/ml) or without apoE for 16 h under culture conditions. Cellular proteoglycans were determined as described under “Materials and Methods.”

| Treatment | Endothelial cells | J774 macrophages | Fibroblasts |
|-----------|------------------|------------------|-------------|
| None      | 10,490 ± 442     | 34,490 ± 3,260   | 15,790 ± 297|
| ApoE      | 32,902 ± 990     | 36,050 ± 4,422   | 15,610 ± 1,120|

PG sulfation in vivo, 35SO4 incorporation was determined in WT and apoE-null mice. 4-week-old mice were injected with 35SO4, and incorporation into PG was determined after 4 h as described under “Materials and Methods.” 35SO4 incorporation was not different in livers of WT and apoE-null mice (Fig. 9). However, hearts (containing part of the aorta) from apoE-null mice had ~40% reduction in 35SO4 incorporation compared with hearts from WT mice. These data suggest that apoE also stimulates sulfation of PG in vivo.
DISCUSSION

Loss of endothelial HS has been postulated to lead to several pathological events, in particular to events related to atherosclerosis (15–19, 27–31). These include 1) altered endothelial permeability, 2) increased cell migration through blood vessel walls, 3) thrombin generation, 4) increased monocyte binding to the subendothelial matrix, 5) increased lipoprotein retention, 6) increased SMC proliferation, and 7) increased susceptibility to bacterial infection. HS has also been shown to inhibit matrix metalloproteinase activity (32), involved in plaque rupture (33), and to regulate the bioavailability of basic fibroblast growth factor activity (34). Agents that decrease endothelial HSPG include lipopolysaccharide and tumor necrosis factor-α (35), homocysteine (36), lysolecithin, and oxidized LDL (18, 19).

Thus, a decrease in HS may be a general inflammatory reaction.

The present studies show that HDL increases endothelial HS and thus could decrease the occurrence of the above mentioned events. HDL has several anti-atherogenic effects, and epidemiological studies inversely correlated HDL with atherosclerosis (37). HDL facilitates reverse cholesterol transport from peripheral tissues to liver (38). In addition, platelet-activating factor acetyl hydrolase and paraoxonase enzymes associated with HDL can metabolize and reduce the content of biologically active oxidized lipids in oxidized LDL (39, 40). Our current results show yet another mechanism by which HDL can be anti-atherogenic, i.e. by increasing sulfation of endothelial HSPG.

HDL increased both glucosamine and sulfate incorporation into PG without significantly affecting the total core proteins. Although total core proteins have not changed based on the elution of [3H]leucine-labeled proteins from DEAE-cellulose, it is conceivable that HDL increased specific PG core proteins which remains to be determined. The ratio of sulfate to glucosamine was increased by HDL, suggesting that the GAG were more sulfated in HDL-treated endothelial cells. The increase in sulfation does not appear to be due to apoE binding to HS and preventing its degradation because incubation of 35SO4-labeled endothelial cells with HDL did not prevent degradation. Thus, HDL treatment may have increased sulfation by affecting sulfotransferrases. Several enzymes involved in the sulfation of HSPG have been characterized (41–45). These include glucosamine N-acyltransferases and O-sulfotransferases and uronic acid 2-O sulfotransferase. It is conceivable that HDL increased the activities of one or more of these enzymes. Regulation of sulfation and sulfotransferases in endothelial cells is a poorly studied area. This is surprising considering the several known anti-atherogenic effects of endothelial heparin and HS. Evidence has recently been put forward that a deficiency of endogenous heparin or heparin-like substances predisposes to atherosclerosis (46, 47), and heparin administration has been shown to increase sulfation of endothelial HS (48).

Subendothelial HS contains substantial amounts of highly sulfated blocks (referred to as heparin-like) (49, 50). These are resistant to heparinase (heparinase III) digestion. Data in Figs. 4 and 5 support the conclusion that HDL increased sulfated HS. Antithrombin binding is largely restricted to heparin chains containing glucosamine N- and 3-O sulfates (51). In our experiments antithrombin binding was increased to matrix prepared from HDL-treated cells, suggesting an increase in GlcNSO3(3-O-SO3)-heparins. Second, HS prepared from HDL-treated matrix inhibited SMC proliferation, suggesting an increase in HS sequences containing 2-O sulfates (25). An increase in 2-O iduronic acid-containing HS was also confirmed by increased binding of lipoprotein lipase (1.6-fold, not shown).

Experiments with HDL prepared from apoE and apoA-I knockout mice suggest that apoE is required for stimulation of sulfation. This was confirmed further by apoE add-back experiments and apoE emulsion experiments. Lipid-free apoE also stimulated endothelial HS production. It is, however, conceivable that apoE acquired cellular lipid during the 16-h incubation. Nevertheless, these data clearly show that apoE is required for HS stimulation. Although containing apoE, VLDL failed to stimulate HS. Whether this is because of conformational changes in VLDL apoE remains to be determined.

Although apoE is known to play a key role in lipoprotein clearance, recent studies from several groups indicate that its anti-atherogenic effects go beyond its role in remnant clearance. Shimano et al. (52) and Bellotta et al. (53) expressed apoE in the vessel and decreased atherosclerosis in apoE-null mice.
without significant changes in plasma lipoproteins. Recently, Fazio et al. (54), by transplanting apoE-null macrophages into normal C57BL6 mice, increased atherosclerosis. How apoE protects the vessel wall from accumulating lipoproteins is not clear. This may in part be the result of its ability to facilitate removal of cholesterol from the cells of arterial walls (37).

Other studies have also suggested possible anti-atherogenic roles for apoE-HDL, including inhibition of lipase-mediated LDL retention (55) and inhibition of platelet aggregation through the nitric oxide pathway (56). Studies from humans as well as mice suggest that low apoE as well as apoE-HDL are important risk factors for vascular disease (57–60). Our observation that apoE-HDL increases sulfation of HS offers an alternative explanation for the anti-atherogenic effects of vascular/macrophage apoE. Atherosclerotic vessels have decreased HS, and we showed that removal of subendothelial HSPG resulted in a 2–10-fold increase in the binding of atherogenic lipoproteins such as lipoprotein(a) and monocyte-macrophages (18, 19). Consistent with this, our present data show that increasing HSPG by HDL treatment decreased the number of monocytes binding to the subendothelial matrix. In addition, an apoE-HDL-mediated increase in vascular HSPG can prevent SMC proliferation in the subendothelial space.

The mechanism of apoE-HDL-mediated stimulation of sulfation is not clear. ApoE is known to affect cell signaling by increasing CAMP and cGMP levels (56). Preliminary experiments showed that 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor (1–5 mM), did not inhibit apoE actions (not shown). It appears that the apoE-mediated effects are cell-specific as no increase in $^{35}S$O$_4$ incorporation was observed in either macrophages or fibroblasts. Data from WT and apoE-null mice further support this. Hearts (with proximal aorta) but not livers showed a significant decrease in $^{35}S$O$_4$ incorporation in apoE-null mice. This may be caused by differences in the tissue metabolism of lipoproteins or endothelial cell heterogeneity (61). However, further experiments are needed to prove that the HS decrease is a direct effect of apoE deficiency. It is conceivable that vascular cell-specific surface molecules mediate apoE actions. ApoE has several known receptor molecules, including LDL receptor, LDL-receptor related protein, VLDL receptor, and proteoglycans. Endothelial cells are not known to express LDL-receptor related protein (62). Our data showed that LDL receptor antibody, excess LDL or VLDL did not inhibit apoE-mediated HS stimulation. RAP, a LDL receptor family antagonist, inhibited apoE actions at high doses. VLDL receptor, which is abundant on endothelial cells, appears to be one potential candidate for mediating apoE actions (62). It is also conceivable that RAP, by direct interaction with cell surface HSPG, inhibited apoE binding (63). However, RAP binding to HS aggregating protein is controversial (64). Heparin also inhibited apoE actions, suggesting that HSPG, either directly or indirectly facilitate binding to RAP-sensitive receptors and are involved in the mediation of apoE actions. Experiments with apoE-null mice also showed decreased $^{35}S$O$_4$ incorporation only in heart but not liver. Although this is surprising considering the fact that liver is an endothelial cell-rich organ, one possible reason for this is the lack of VLDL receptors or other potential RAP-sensitive receptors.

In summary, our data show that apoE HDL enhances sulfation of endothelial PG and suggest a novel mechanism by which apoE can be anti-atherogenic. These effects were seen within the physiological concentrations of HDL and within the levels of apoE found in HDL (24). Our data, in addition, offer an alternative explanation for the anti-atherogenic effects of macrophage apoE. After synthesis and secretion from macrophages, apoE or apoE particles can act locally and stimulate endothelial HSPG. Increased HSPG can inhibit subsequent accumulation of lipoproteins and monocytes and inhibit subendothelial SMC proliferation. Thus, identification of specific apoE peptides that can stimulate endothelial cell heparin production may have important therapeutic application.

REFERENCES

1. Wight, T. N. (1989) Arteriosclerosis 9, 1020–1026
2. Rosenberg, R. D., Shewok, N. W., Liu, J., Schwartz, J. J., and Zhang, L. (1997) J. Clin. Invest. 99, 2063–2070
3. Pillarisetti, S., Paka, L., Sasaki, A., Yin, B., Parthasarathy, N., Wagner, W. D., and Goldberg, I. (1997) J. Biol. Chem. 272, 15753–15760
4. Vlodavsky, I., Miao, H. Q., Medal, B., Danagher, P., and Ron, D. (1996) Cancer Metastasis Rev. 15, 177–186
5. de Agostini, A. I., Watkins, S. C., Slayter, H. S., Youssoufian, H., and Rosenberg, R. D. (1990) J. Biol. Chem. 265, 1293–1304
6. Wight, T. N. (1985) Fed. Proc. 44, 381–385
7. Wagner, W. D. (1985) Ann. N. Y. Acad. Sci. 454, 52–68
8. Srinivasan, S. R., Rathakrishnamurthy, B., Vijayapal, P., and Berenson, G. S. (1991) Adv. Exp. Med. Biol. 286, 19–28
9. Hoff, H. F., and Wagner, W. D. (1986) Atherosclerosis 61, 231–236
10. Hollman, J., Schmidt, A., von Bassewitz, D., and Buddecke, E. (1989) Arteriosclerosis 9, 154–158
11. Volker, W., Schmidt, A. Oortmann, W., Broszey, T., Faber, V., and Buddecke, E. (1990) Eur. Heart J. 11, 29–40
12. Murata, K., and Yokoyama, Y. (1996) Atherosclerosis 78, 69–79
13. Kruse, R., Merten, M., Yoshida, K., Schmidt, A., Volker, W., and Buddecke, E. (1990) Basic Res. Cardiol. 91, 344–352
14. Murata, K. (1985) Stroke 16, 687–694
15. Rosenberg, R. D. (1989) Adv. Med. 87, 28–98
16. Cloves, A. W., and Karonowsky, M. J. (1977) Nature 265, 625–626
17. Vlodavsky, I., Miao, H. Q., Atzmon, R., Levi, E., Zimmermann, J., Bar-Shavit, R., Peretz, T., and Ben-Sasson, S. A. (1995) Thromb. Haemostasis 74, 534–540
18. Pillarisetti, S., Obunike, J. C., and Goldberg, I. J. (1995) J. Biol. Chem. 270, 29760–29765
19. Pillarisetti, S., Paka, S., Obunike, J., Berglund, L., and Goldberg, I. J. (1995) J. Clin. Invest. 100, 867–874
20. Al-Haideri, M., Goldberg, I. J., Galeano, N. F., Gleeson, A., Vogel, T., Gorecki, M., Surley, S. L., and Deckelbaum, R. J. (1997) Biochemistry 36, 12766–12772
21. Pillarisetti, S., Klein, M. G., and Goldberg, I. J. (1992) J. Biol. Chem. 267, 16517–16522
22. Edwards, I. J., Xu, H., Obunike, J. C., Goldberg, I. J., and Wagner, W. D. (1995) Arterioscler. Thromb. Vasc. Biol. 1991, 400–409
23. Stina, M. F., Pillarisetti, S., Sasaki, A., and Goldberg, I. J. (1993) J. Lipid Res. 34, 1853–1861
24. Gotto, A. M., Jr., Pownall, H. J., and Havel, R. J. (1986) Methods Enzymol. 128, 3–41
25. Slight, A., Yoshida, K., and Buddecke, E. (1992) J. Biol. Chem. 267, 1924–1927
26. Parthasarathy, N., Goldberg, I. J., Sivaram, P., Mulloy, B., Flory, D. M., and Wagner, W. D. (1994) J. Biol. Chem. 269, 22391–22396
27. Richardson, M., Gerrity, R. G., Alavi, M. Z., and Moore, S. (1982) J. Clin. Invest. 69, 1987–1993
28. Gallis, Z., Sukhova, G., Lark, M., and Libby, P. (1994) J. Clin. Invest. 94, 2493–2503
ApoE HDL Stimulates Endothelial Heparan Sulfate

46. Engelberg, H. (1991) *Semin. Thromb. Hemostasis* 17, (Suppl. 1) 5–8
47. Engelberg, H. (1997) *Semin. Thromb. Hemostasis* 23, 159–166
48. Pinhal, M. A., Santos, I. A., Silva, J. F., Dietrich, C. P., and Nader, H. B. (1995) *Thromb. Haemostasis* 74, 1169–1174
49. Nader, H. B., Dietrich, C. P., and Colburn, P. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 3565–3569
50. Pye, D. A., and Kumar, S. (1995) *Biochim. Biophys. Acta* 1266, 235–244
51. Razi, N., and Lindahl, U. (1995) *J. Biol. Chem.* 270, 11267–11275
52. Shimano, H., Ohsuga, J., Shimada, M., Namba, Y., Gotoda, T., Harada, K., Katsuki, M., Yazaki, Y., and Yamada, N. (1995) *J. Clin. Invest.* 95, 469–476
53. Bellota, S., Mahley, R. W., Sanan, D. A., Murata, J., Newland, D. L., Taylor, J. M., and Pitas, R. E. (1995) *J. Clin. Invest.* 96, 2170–2179
54. Fazio, S., Bahaev, V. R., Murray, A. B., Hasty, A. H., Carter, K. J., Gleaves, L. A., Atkinson, J. B., and Linton, M. F. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 4647–4652
55. Saxena, U., Ferguson, E., and Bisgaier, C. L. (1993) *J. Biol. Chem.* 268, 14812–14819
56. Riddell, D. R., Graham, A., and Owen, J. S. (1997) *J. Biol. Chem.* 272, 89–95
57. Skinner, E. R. (1994) *Curr. Opin. Lipidol.* 5, 241–247
58. Couderc, R., Mahieux, F., Bailleul, S., Fenelon, G., Mary, R., and Fermanian, J. (1993) *Stroke* 24, 661–664
59. Wilson, H. M., Patel, J. C., Russell, D., and Skinner, E. R. (1993) *Clin. Chem. Acta* 220, 175–187
60. de Silva, H. V., Mas-Oliva, J., Taylor, J. M., and Mahley, R. W. (1994) *J. Lipid Res.* 35, 1297–1310
61. Rajotte, D., Arap, W., Hagedorn, M., Keivunen, E., Pasqualini, R., and Ruoslahti, E. (1998) *J. Clin. Invest.* 102, 430–437
62. Moestrup, S. K., Glioman, J., and Pallesen, G. (1992) *Cell Tissue Res.* 269, 3–10
63. Ji, Z. S., and Mahley, R. W. (1994) *Arterioscler. Thromb.* 14, 2025–2031
64. Vassiliou, G., and Stanley, K. K. (1994) *J. Biol. Chem.* 269, 15172–15178