# Lipoprotein lipase enhances the interaction of low density lipoproteins with artery-derived extracellular matrix proteoglycans

**Iris J. Edwards,[1] Ira J. Goldberg,* John S. Parks, Hongzhi Xu, and William D. Wagner**

Department of Comparative Medicine, Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040, and Department of Medicine,* Columbia University, New York, NY 10032

**Abstract** The association of plasma low density lipoproteins (LDL) with arterial proteoglycans (PG) is of key importance in LDL retention and modification in the artery wall. Lipoprotein lipase (LpL), the rate-limiting enzyme for hydrolysis of lipoprotein triglyceride, is known to bind both LDL and arterial PG. In the presence of LpL, cellular internalization and degradation of LDL is enhanced by a pathway initiated by interaction of LDL with a cell surface heparan sulfate proteoglycan. To determine whether LpL enhances the binding of LDL to arterial chondroitin sulfate (CS)PG and dermatan sulfate (DS)PG, the major extracellular PG of the artery wall, a microtiter plate assay was used to study LpL-PG-LDL interactions. Binding of LDL to both CSPG and DSPG was increased in the presence of LpL but differential effects were seen for the two PG. LpL enhanced the binding of LDL to CSPG a maximum of 20% and to DSPG a maximum of 40%. Heparin displacement of PG binding suggested a greater binding strength for DSPG-LpL-LDL with 0.25 μg heparin required to displace 50% of DSPG compared to 0.01 μg to displace 50% of CSPG. The greater enhancement of DSPG-LDL interaction by LpL is of particular interest since increases in DSPG correlate with the accumulation of aortic cholesterol. These data suggest that lipoprotein lipase may enhance the interaction of plasma low density lipoprotein with arterial chondroitin sulfate proteoglycan and dermatan sulfate proteoglycan and thus facilitate low density lipoprotein retention in the artery wall.—**Edwards, I. J., I. J. Goldberg, J. S. Parks, H. Xu, and W. D. Wagner.** Lipoprotein lipase enhances the interaction of low density lipoproteins with artery-derived extracellular matrix proteoglycans. *J. Lipid Res.* 1993. **34:** 1155–1163.

**Supplementary key words** triglyceride • atherosclerosis • glycosaminoglycan • proteoglycan • dermatan sulfate • chondroitin sulfate • heparan sulfate

Lipoprotein lipase (LpL), the enzyme responsible for hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL), is present in both normal arterial tissue and in atherosclerotic plaque (1), where it is synthesized by both smooth muscle cells (2) and macrophages (2, 3). In rabbit aortas, LpL activity has been shown to increase with increasing cholesterol content (1). This led to a hypothesis that LpL may promote atherosclerosis by the production of cholesterol ester-rich remnant particles that deposit in the artery wall. Recently, a second major role for LpL in atherogenesis has been suggested by Saxena et al. (4), who observed that LpL-mediated hydrolysis of VLDL was associated with increased transport of low density lipoprotein (LDL) across endothelial cell monolayers and enhanced LDL retention in the subendothelial matrix. A proposed mechanism for LpL-mediated LDL retention involved the formation of a complex between LDL, LpL, and the heparan sulfate-proteoglycan (HSPG) of the subendothelial matrix. Three recent reports have indicated that LpL-induced binding of LDL to cell surface HSPG resulted in enhanced internalization and degradation of the LDL in fibroblasts (5–7), HepG2 cells (5, 6), and macrophages (7). The LDL uptake pathway involving HSPG may be an important mechanism, independent of the LDL receptor, that allows lipid accumulation in arterial cells.

The association of LDL with arterial proteoglycans (PG) is a key factor in LDL retention and modification in the arterial wall. More LDL is retained in atherosclerosis-prone segments of rabbit aorta (8), presenting a greater potential for LDL modification by oxidative enzymes. Chemically modified LDL has been identified in atherosclerotic rabbit (9, 10) and human arteries (10–12). Extraction of LDL from atherosclerotic plaques resulted in co-isolation of glycosaminoglycans (GAG), the carbohydrate moiety of PG (11, 12), suggesting that retention of LDL on PG may promote atherosclerosis.

---

Abbreviations: LpL, lipoprotein lipase; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PG, proteoglycan(s); DS, dermatan sulfate; CS, chondroitin sulfate; HS, heparan sulfate; apoB, apolipoprotein B; GAG, glycosaminoglycan(s).

[1]To whom reprint request should be addressed.

Of the total artery PG, HSPG is a minor component being associated primarily with cell and basement membranes. Chondroitin sulfate-proteoglycan (CSPG) and dermatan sulfate-proteoglycan (DSPG) are the major extracellular PG of the artery wall, and both are increased during atherosclerosis development (for review, see ref. 13). Both have been implicated in atherogenesis by their ability to bind plasma LDL through ionic associations between the negatively charged GAG chains of the PG and positively charged amino groups of the LDL apolipoprotein (apo)B (14). Several studies have shown that in vitro interaction of plasma LDL with mixtures of CSPG and DSPG potentiated LDL uptake and accumulation in macrophages by internalization of either particulate complexes of PG-LDL (15, 16) or internalization of LDL oxidatively modified after transient interaction with arterial PG (17). Thus, factors that potentiate LDL interaction with extracellular matrix PG may be important in LDL entrapment and accumulation in the arterial wall.

The present study was based on the hypothesis that LpL facilitates LDL retention in the artery wall by increasing the binding of LDL to arterial CSPG and DSPG. The results indicate that LpL binds to both CSPG and DSPG, thereby enhancing the interaction of these PG with LDL.

## METHODS

### Lipoprotein preparation

The animals used to provide LDL for these studies have been described previously (18). They were cynomolgus monkeys fed a diet containing 40% of calories as fat derived 50% from lard and 50% from egg yolk with 0.20 mg cholesterol/kcal. Complete details of these diets have been published (19). Blood samples were taken from the femoral vein of two animals after an 18-h fast and sedation with ketamine hydrochloride (10 mg/kg). Blood was placed in chilled tubes containing 0.1% EDTA and 0.02% $NaN_3$ (final concentration), pH 7.4. LDL was isolated from plasma by ultracentrifugation and high performance liquid chromatography (HPLC) on a Superose 6B column, and chemical composition of LDL was measured by standard procedures (19). The LDL cholesterol and protein concentrations were 3.15 mg/ml and 1.39 mg/ml, respectively. LDL was stored at 4°C under argon.

### PG preparation

The CSPG preparation used in these studies has been described previously (18). Briefly, it was obtained from normal artery of thoracic aortas from two cynomolgus monkeys by extraction with 4.0 M GdnHCl and 0.05 M sodium acetate (pH 4.5) containing protease inhibitors (20) at 15 ml/g wet tissue. After a 24-hour extraction at 4°C, PG were isolated by chromatography on a Sepharose CL-4B column using 4.0 M GdnHCl and 0.05 M sodium acetate (pH 5.8) as the elution buffer. As previously shown with human (21) and pigeon arteries (22), two major PG fractions were separated, one eluting near the column $V_o$ (which contained predominantly CSPG), and the other eluting at a $K_{av}$ of 0.4, which contained predominantly DSPG. The fractions comprising the two PG were pooled and further purified by cesium chloride isopynic gradient centrifugation as previously described (21, 22). The bottom two-fifths of each gradient were collected, analyzed for hexuronic acid (23) and protein (24) and stored at −70°C. The CSPG preparation contained 130 μg/ml hexuronic acid and 149 μg/ml protein and based on SDS gels had an estimated $M_r$ of $2 \times 10^6$. The DSPG preparation which had an approximate $M_r$ of 250 kDa, contained 104 μg/ml hexuronic acid and 85 μg/ml protein. The DSPG preparation reacted with both anti-decorin (LF-30) and anti-biglycan (LF-51) antibodies (gift from Dr. Larry Fisher, NIH).

### Biotin conjugation of PG

Aliquots of CSPG and DSPG were biotin-conjugated according to published procedures (25). To confirm the specificity of biotin labeling, PG preparations were electrophoresed on 3–20% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride-based membranes (Bio-Rad) for 3 h at 30 volts, then for 1 h at 100 volts at 4°C. Membranes were washed in Tris-saline (0.9% NaCl, 10 mM Tris), pH 7.4, blocked for 1 h at 26°C with 5% bovine serum albumin (BSA) in Tris-saline, and incubated with streptavidine peroxidase (10 μg/ml) for 2 h at 26°C. A substrate of 8 mg 4-chloro-1-naphthol in 2 ml methanol and 8 μl 30% $H_2O_2$ in 20 ml Tris-saline was used to develop a color reaction in approximately 15 min. The reaction was stopped with distilled water when background color first started to appear. Membranes were dried and scanned using a laser densitometer. Based on the areas under peaks, contaminating proteins accounted for <5% of the labeled CSPG and <15% of the labeled DSPG.

To determine relative levels of biotinylation, protein was measured in each preparation and serial dilutions based on protein concentration were analyzed for extent of biotinylation by a dot-blot assay. Biotinylated BSA was used as a standard and peroxidase-conjugated streptavidin (Sigma) was used to detect and measure bound PG-biotin and BSA-biotin. Membranes were scanned using a laser densitometer, and the relative biotinylation of the two PG preparations was calculated by comparing them to BSA. It was determined that per μg protein, CSPG was biotinylated 3.7-fold greater than DSPG.

### LpL preparation

LpL was prepared from fresh unpasteurized bovine milk using heparin-agarose (Bio-Rad) affinity chromatog-

raphy following the method of Socorro, Green, and Jackson (26) as previously described (27). The purity of the enzyme was established by SDS polyacrylamide gel electrophoresis using a 4–30% gradient gel. Enzyme activity was determined (27) and aliquots were stored at −70°C. Biotin-conjugated LpL was prepared according to the procedure previously described for biotin-conjugated PG. Biotinylated LpL was purified by heparin affinity chromatography prior to use.

### Assay for PG-LDL-LpL interactions

A modification of the method of Christner and Baker (25) for assessment of PG-LDL binding was used to assess PG-LpL-LDL interactions. LDL in 0.14 M sodium chloride, 0.01 M sodium phosphate (PBS), pH 7.0, containing 0.01% ethylenediamine tetraacetic acid (EDTA) was immobilized by passive adsorption to polyvinyl chloride microtiterplates (Falcon) for 18 h at 4°C. After removal of unbound LDL by inverting the plates, the wells were washed three times with PBS, blocked with 5% BSA in PBS for 1 h at 26°C, and washed three times with PBS. Wells were washed once with 10 mM Tris, 50 mM NaCl, 5 mM $CaCl_2$, and 0.05% BSA (interaction buffer) before adding LpL in the same buffer. After a 1-h incubation at 26°C, LpL was removed and biotinylated PG in interaction buffer was added. In some experiments, competing LDL or PG were diluted in interaction buffer and added to wells immediately after the addition of biotinylated PG. All volumes were adjusted to give a standard well volume of 100 μl. PG-LDL interaction was measured after 1 h at 26°C. Unbound PG was removed by inverting the plate, and wells were washed three times with 50 mM Tris, 90 mM NaCl, 5 mM $CaCl_2$, 0.05% BSA (substrate buffer) before adding streptavidin peroxidase (10 μg/ml substrate buffer) for 2 h at 26°C. Wells were washed three times with PBS. The chromogen o-dianisine (Sigma), 1% in methanol–water 1:1 was prepared fresh before each use to develop a color reaction (28). A substrate solution of 0.006% hydrogen peroxide, 0.008% o-dianisidine in 0.1 M sodium phosphate-sodium citrate buffer, pH 5.0, was added to the wells and color was developed in the dark for 30 min. The reaction was stopped by addition of 12.5 μl of 2 N HCl per well. The plates were vortexed to mix the reaction medium and absorbance was measured at 405 nm on a Dynatech MR 580 microtiter plate reader.

## RESULTS

The first set of experiments was designed to optimize assay conditions for the binding of PG and LDL.

### Assessment of LDL binding to microtiter wells

Initial experiments were completed to determine the effect of LDL concentration on LDL bound to the microtiter wells and on PG binding. For these studies, increasing amounts of LDL were added to wells and incubated for 18 h at 4°C. After removal of unbound LDL, biotinylated CSPG was added to the LDL-coated wells for 1 h at 26°C and bound PG was measured. The saturation kinetics for LDL in the assay are shown in **Fig. 1.** Maximum binding was reached with 1 μg LDL (as protein) per well. Similar results were obtained when LDL coating conditions were changed to 2 h at 37°C (data not shown). Therefore, all subsequent experiments used dishes coated with LDL for 16 h at 4°C, as LDL is more stable at the lower temperature. An enzymatic assay for total cholesterol (Boehringer Mannheim Diagnostics) indicated that when 5 μg LDL (as protein) was added per well, a maximum of 0.46 ± 0.02 μg LDL total cholesterol (0.20 μg LDL protein) was bound. When either HDL or excess LDL was added to the LDL coated plate at the same time as the PG, only LDL competed for binding (data not shown).

### Assessment of CSPG binding to LDL-coated microtiter wells

When the concentration of coating LDL was held constant and the CSPG concentration varied, the binding curve shown in **Fig. 2** was generated. Binding began to plateau at approximately 0.2 μg PG. Based on these data, a subsaturating level of 0.1 μg PG was used in subsequent experiments to determine the effect of LpL on PG-LDL binding.

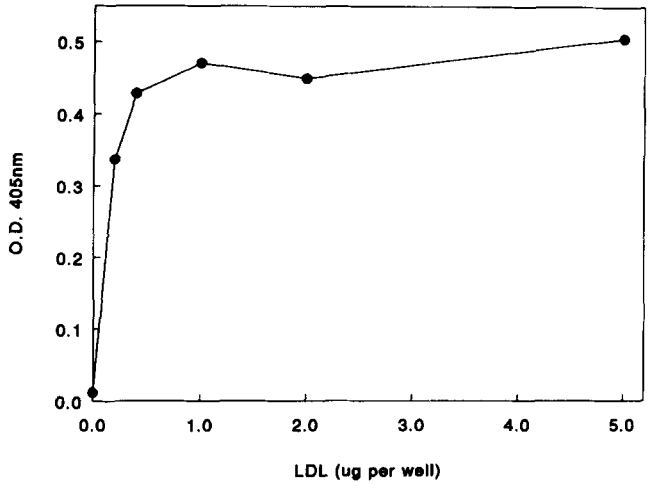


**Fig. 1.** LDL binding to microtiter plate wells. LDL (0–5 μg as protein per well) was diluted in 100 μl phosphate-buffered saline (PBS)-0.01% ethylenediaminetetraacetic acid (EDTA), and adsorbed to microtiter plate wells for 18 h at 4°C. Wells were washed three times with PBS and blocked with 5% bovine serum albumin (BSA) in PBS before adding 0.1 μg biotin-conjugated chondroitin sulfate-proteoglycan (CSPG) in 100 μl interaction buffer for 1 h at 26°C. After removing proteoglycans (PG) by washing with PBS, bound PG was measured using a streptavidin-peroxidase detection system (described in Methods). Points are means of triplicate wells. SEM ranged from 0.004 to 0.017.

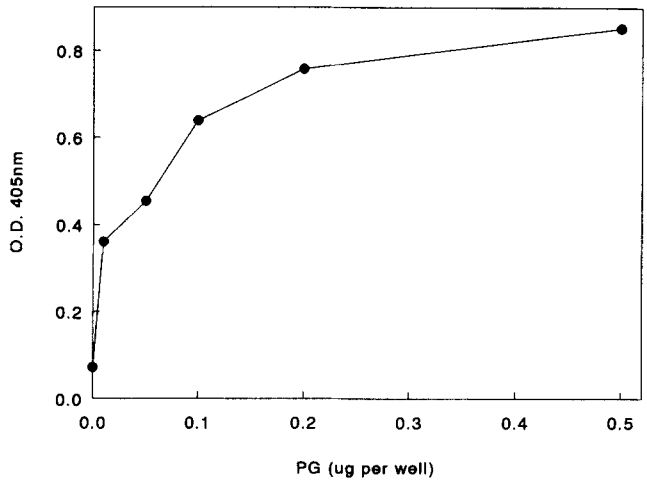


**Fig. 2.** PG binding to LDL-coated wells. LDL (5 μg as protein per well) was adsorbed to microtiter plate wells as described in Fig. 1 and blocked with 5% BSA in PBS before adding 0–5 μg biotin-conjugated CSPG for 1 h at 26°C. Unbound PG was removed by washing with PBS and bound PG was measured by a streptavidin-peroxidase detection procedure (described in Methods).

### Assessment of LpL binding to LDL and CSPG

The next studies were designed to demonstrate that LpL binds to both LDL and CSPG. **Fig. 3** illustrates the binding of biotinylated LpL to microtiter plate wells previously incubated with 5 μg LDL for 16 h at 4°C. The addition of increasing levels of LpL resulted in increased binding to LDL, with near saturation around 0.5 μg LpL.

To assess the interaction of LpL and CSPG, the wells of a microtiter plate were coated with varying concentrations of non-biotinylated LpL. Biotinylated CSPG (0.1 μg) was then added to the LpL-coated wells and allowed to bind for 1 h at 26°C. As shown in **Fig. 4,** as increasing amounts of LpL were added to the wells, increasing amounts of CSPG were bound. When plates were coated with either 3 μg or 6 μg BSA, absorbance values of 0.05 and 0.08 were obtained, respectively, indicating that LpL binds to CSPG and not nonspecifically to any protein.

### Enhancement of LDL binding to CSPG and DSPG in the presence of LpL

The preceding studies indicated that LpL bound to both LDL and CSPG. To test the hypothesis that the binding of LpL to LDL and PG increases LDL-PG associations, the effect of LpL on CSPG-LDL interaction was studied. In addition, the effect of LpL on the interaction of LDL with DSPG, the second most abundant arterial PG, also was assessed in the presence of LpL. LpL (0.1 to 1.0 μg) was added to LDL-coated wells for 1 h at 36°C. Unbound LpL was removed and 0.1 μg CSPG or DSPG was then added. As shown in **Fig. 5,** the association of both CSPG and DSPG with LDL was increased in the presence of LpL with a greater increase observed in DSPG binding. CSPG and DSPG isolated from pigeon arteries (22) displayed binding characteristics similar to the monkey PG. Maximum enhancement was not achieved in this experiment because PG levels were limiting.

In the next study, a range of PG concentrations was examined to compare the binding of the two different PG to LDL in the presence or absence of LpL. Binding of CSPG and DSPG to LDL in the absence of LpL plateaued at approximately 0.1 μg PG and reached saturation at about

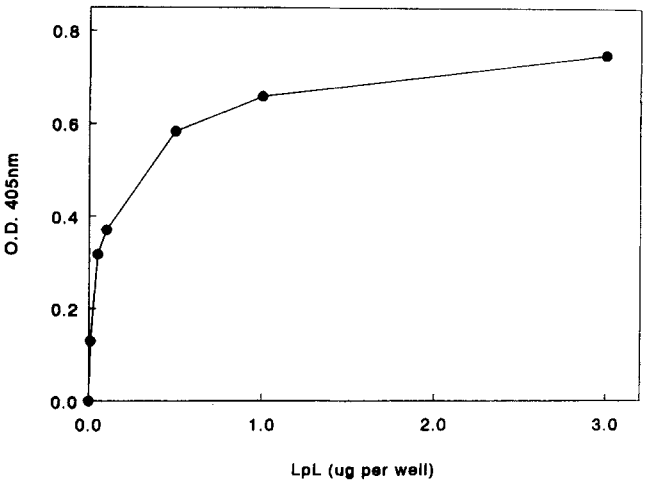


**Fig. 3.** Lipoprotein lipase (LpL) binding to LDL-coated wells. LDL (5 μg as protein per well) was adsorbed to microtiter plate wells as described in Fig. 1. Wells were blocked with 5% BSA in PBS for 1 h at 26°C and washed three times with PBS before adding 0–3 μg biotinylated LpL for 1 h at 37°C. After removing unbound LpL by washing with PBS, bound LpL was measured by the same streptavidin-peroxidase detection procedure noted in Fig. 2.

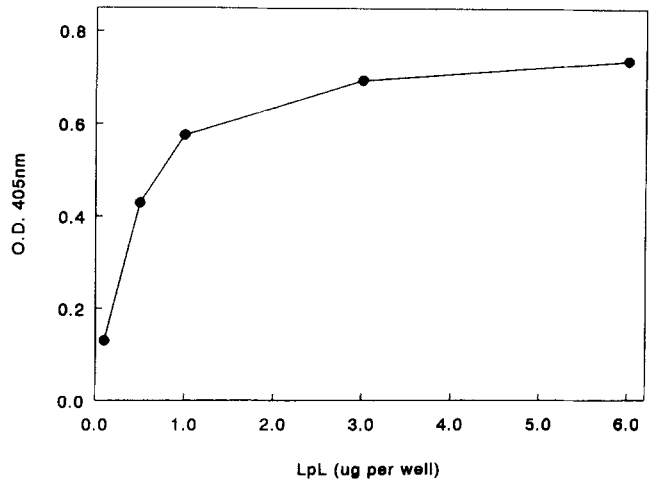


**Fig. 4.** CSPG binding to LpL. LpL (0–6 μg per well in PBS) was added to microtiter plate wells in 100-μl volumes at 4°C for 18 h. BSA (3 μg or 6 μg in PBS) was added to control wells. After removing LpL, wells were blocked with 5% BSA in PBS for 1 h at 26°C and washed three times with PBS. Biotin-conjugated CSPG (0.1 μg/100 μl interaction buffer) was added to each well for 1 h at 26°C. After removing unbound PG, bound PG was measured as noted in Fig. 2.

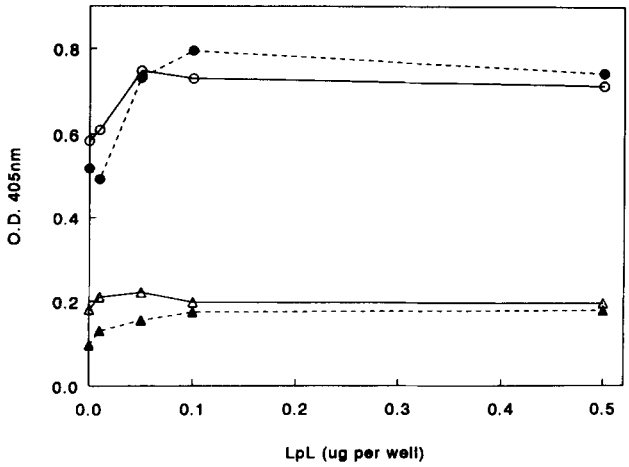


**Fig. 5.** Association of PG and LDL in the presence of LpL. LDL (5 μg as protein per well) was used to coat microtiter plate wells as described in Fig. 1. After removing unbound LDL, wells were blocked with 5% BSA in PBS and washed three times with PBS before incubation with 0.1–0.5 μg LpL in interaction buffer for 1 h at 37°C. Unbound LpL was removed, plates washed three times with PBS, and biotin-conjugated CSPG or DSPG (0.1 μg in 100 μl interaction buffer) added for 1 h at 26°C. After removing unbound PG, bound PG was measured as noted in Fig. 2. Points are means of duplicate wells and adjusted to account for differences in biotinylation of CSPG and DSPG. CSPG, monkey (closed triangles), pigeon (open triangles); dermatan sulfate-proteoglycan (DSPG), monkey (closed circles), pigeon (open circles). Range of agreement of duplicates was 78–98% for monkey CSPG, 89–100% for monkey DSPG, 75–99% for pigeon CSPG, and 93–99% for pigeon DSPG.

0.2 μg (**Fig. 6**). In the presence of LpL, CSPG-LDL binding was enhanced only at pre-saturating concentrations of PG, and the maximum amount of CSPG bound at saturation remained the same. By contrast, LpL enhanced DSPG-LDL binding by increasing the maximum amount of DSPG that could be bound to the LDL at saturation. The increased binding due to the presence of LpL ranged from 0 to 20% for CSPG and 17 to 40% for DSPG.

### Displacement of CSPG and DSPG from LDL-LpL by heparin

Heparin displacement studies were used to compare binding characteristics of CSPG and DSPG to LpL-LDL. To determine relative binding strengths, LpL was bound to an LDL-coated microtiter plate and CSPG or DSPG were added to the wells in the presence of varying concentrations of heparin. As shown in **Fig. 7**, 0.01 μg heparin displaced 50% of CSPG; however, 0.25 μg heparin was required to displace the same amount of DSPG, indicating a greater binding strength for DSPG. Based on previous studies of PG-LpL interaction (29), the model that has been proposed to date involves LpL binding exclusively to GAG. The inability of heparin, a GAG devoid of core protein, to compete totally with DSPG (Fig. 7) suggests that interactions in addition to the GAG, perhaps with the DSPG core protein, may be possible.

If LpL were enhancing DSPG core protein-LDL interactions, different heparin displacement kinetics would be observed in the presence and absence of LpL. As shown in **Fig. 8**, heparin competition for DSPG binding to LDL produced similar displacement curves in the presence or absence of LpL, although more DSPG was bound in the presence of LpL at all heparin concentrations. This suggested that DSPG was associating with LpL at a site similar to heparin and that only the DSPG GAG chains were involved in the interaction. Any interactions between the DSPG core protein and LDL were not directly affected by LpL. Insufficient unlabeled PG was available for a complete analysis of displacement kinetics by CSPG and DSPG; however, a preliminary experiment indicated that

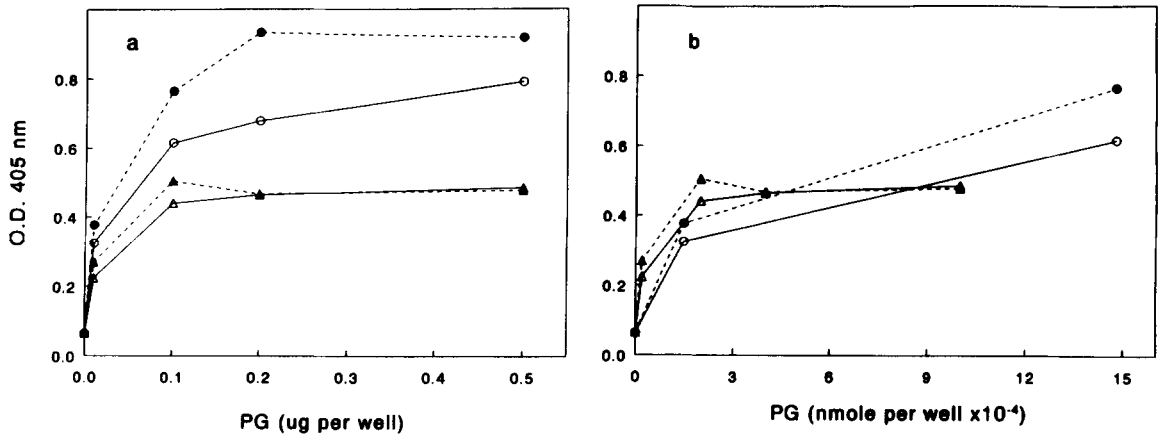


**Fig. 6.** Effect of CSPG (open triangles), LpL + CSPG (closed triangles), DSPG (open circles), and DSPG + LpL (closed circles) concentrations on enhanced LDL binding based on mass (a) or molar (b) amounts of PG. Experimental procedure was same as that described for Fig. 5 except that LpL was either absent or present at 0.1 μg and biotinylated PG levels varied from 0 to 0.5 μg. Results are means of duplicate wells and have been adjusted to account for differences in relative biotinylation of CSPG and DSPG. Agreement between duplicates ranged from 91 to 99%. Molar calculations are based on an estimated $M_r$ of $2 \times 10^6$ daltons for CSPG and 250,000 daltons for DSPG and hexuronic acid comprising 25% of the CSPG and 27% of DSPG.

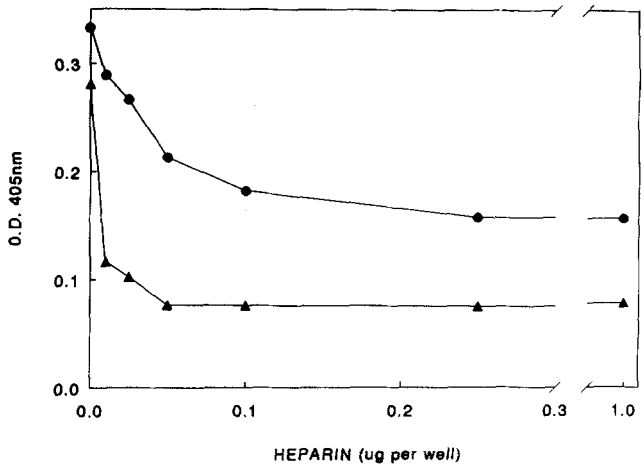


**Fig. 7.** Displacement by heparin of CSPG (triangles) and DSPG (circles) bound to LDL-LpL. Experimental procedure was similar to that described for Fig. 5 except that a constant 0.1 μg LpL and 0.1 μg CSPG or DSPG were used. In addition, 0–0.5 μg heparin (pig mucosa) was added to wells with biotinylated PG. Points are means of duplicate wells. Agreement between duplicates ranged from 79 to 99% for CSPG and from 96 to 99% for DSPG.

1 μg of unlabeled CSPG or DSPG displaced the binding of 0.1 μg biotinylated CSPG to LpL-LDL. Displacement of 0.1 μg biotinylated DSPG was still linear and not complete with 2 μg of either CSPG or DSPG. The results of these studies indicate different binding characteristics for CSPG and DSPG with LpL-LDL. In addition, a substantial amount of bound DSPG was not displaceable by heparin, suggesting non-GAG interactions between DSPG and LDL.

## DISCUSSION

The purpose of these studies was to examine the interactions of LDL, LpL, and arterial CSPG and DSPG to identify a potential mechanism of LDL entrapment in the artery wall. The results provide several new observations. LpL binds both to LDL and to CSPG and DSPG, thus enhancing the interaction of these PG with LDL. Differential effects were observed for the two PG with a greater LpL-induced increase in LDL binding for DSPG than CSPG. Heparin displacement studies identified further binding characteristics: *1)* a greater binding strength for DSPG-LpL-LDL than for CSPG-LpL-LDL; and *2)* whereas CSPG can be almost totally displaced from LDL-LpL by heparin, ~40% of DSPG is not heparin displaceable, indicating that non-GAG interactions with LDL may exist for DSPG.

CSPG and DSPG are the predominant PG of normal artery and play a major role in controlling the structural integrity and permeability of the tissue. Because of their anionic nature and their location in the extracellular matrix, they are prime candidates in a mechanism for entrapment of plasma LDL. Early investigations of PG-LDL interactions focused on GAG, the carbohydrate moiety of PG. When various GAG were bound to LDL under physiological conditions, relative binding affinities of DS > HS > chondroitin-4-sulfate were demonstrated (14). Later, when the structure of the GAG was examined, the charge density and iduronate content were shown to be important factors in LDL binding (30, 31). Studies demonstrating the binding of intact PG to LDL suggested that although the primary interaction of PG with LDL was via the GAG chains, the PG core protein also may play a role by stabilizing the GAG-LDL interaction (22). In the present study, heparin which is a GAG without protein, displaced 88% of CSPG, suggesting that the CSPG-LDL interaction was via the GAG of CSPG with little involvement of the core protein. As the CSPG core protein is heavily glycosylated with at least 20–30 GAG (13), there is probably little potential for LDL-CSPG core protein interaction. For DSPG with only one (decorin) or two (biglycan) GAG chains, interaction of LDL with both GAG and core protein remains a strong possibility. Core proteins of both decorin and biglycan have hydrophobic domains, which theoretically would allow protein-protein interaction with LDL. In the present study only 50% of DSPG was displaced by heparin. This may represent a preferential displacement of either decorin or biglycan or an equal but incomplete displacement of both. Although LpL did not influence the interaction of the DSPG core protein with LDL (Fig. 8), LpL would be a factor in PG core-LDL association as its interaction with the DSPG chains would present increased number of core proteins capable of binding to LDL.

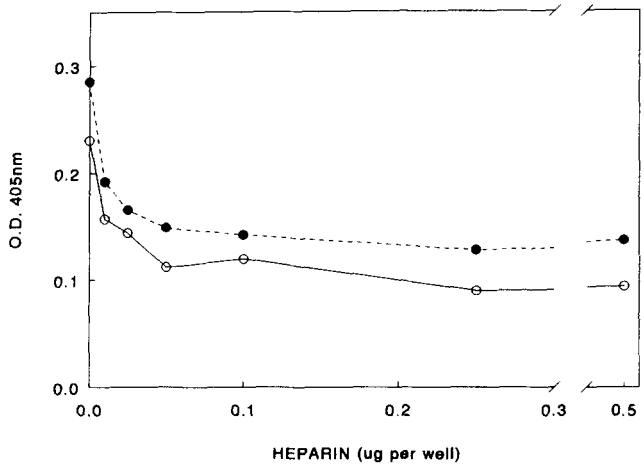


**Fig. 8.** Displacement by heparin of DSPG bound to LDL in the presence (closed circles) and absence (open circles) of LpL. LDL was used to coat microtiter plate wells before adding 0 or 0.1 μg LpL per well as described in Figs. 1 and 5. Biotinylated DSPG (0.1 μg in interaction buffer) was added to wells with 0–0.5 μg heparin (pig mucosa). After 1-h incubation at 26°C, bound biotin-conjugated PG was measured. Points are means of duplicate wells. Agreement between duplicates ranged from 97 to 99%.

Margelin et al. (29) reported LpL binding with a mixed aorta CSPG-DSPG preparation. The present data extend those observations by demonstrating that PG-LpL interaction causes enhanced PG-LDL interaction. In addition, by using purified PG, it was possible to identify a greater effect of LpL on the LDL binding of DSPG compared to CSPG. This finding is of considerable interest, since although both CSPG and DSPG increased during atherosclerosis development (32–37), the most consistent change, relative to starting levels, is a greater increase in DSPG (32–35).

It has been shown previously that LpL binds to a cell surface HSPG on endothelial cells (38), fibroblasts (6), and macrophages (7). HSPG are a heterogeneous family of macromolecules and specific structural features such as carbohydrate sequences, extent and position of sulfation and acetylation are linked to specific functional properties (for review, see ref. 39). The structural determinants for the interaction of HSPG and LpL are unknown. Bengtsson et al. (40) examined the binding of LpL to various GAG and showed relative binding affinities of heparin > HS > DS > CS. Those studies suggested that iduronate residues (which are present in heparin, HS, and DS) were important in the interaction, but that N sulfation (present in heparin and HS) was not. In those studies, a low level of LpL binding (requiring 0.32 M NaCl for dissociation) was also observed for CS, which contains glucuronic acid instead of iduronic acid. The present observations of the binding of intact CSPG and DSPG are in agreement with that report.

The presence of LpL may modify the PG binding site on LDL. Aviram, Bierman, and Chait (41) have shown that incubation of human LDL with LpL depleted LDL triglyceride and produced LDL particles with enhanced uptake resulting in cholesterol esterification in macrophages and smooth muscle cells. They suggested that LDL core reduction resulting from triglyceride depletion may have altered apoB conformation, which facilitated LDL uptake. Since specific segments of apoB are involved in interactions with PG (42), LpL effects that modify apoB conformation may, in turn, affect LDL-PG interactions. Other recent data have demonstrated that LpL increases cellular uptake of non-apoprotein emulsions (7), suggesting that the interaction of LpL and LDL is mediated through LDL phospholipids rather than protein. This also may result in conformational changes in LDL apoB. An alternative mechanism of LpL enhancement of PG-LDL interaction may simply involve the formation of a structural bridge between the two molecules. Recent studies have suggested that enzymatic activity of the lipase is not required for the enhancement of LDL uptake in cells via the pathway involving cell surface HSPG (6, 7).

In summary, based on the experimental results of these and other studies, a model can be proposed for the interactions of CSPG and DSPG with LDL-LpL. Both LDL

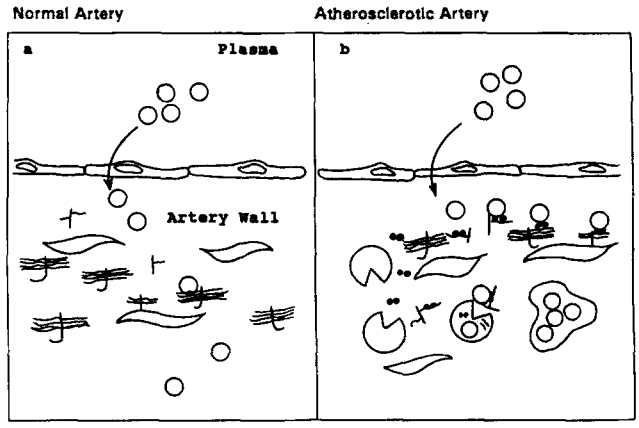


**Fig. 9.** Proposed model for enhanced LDL retention in the artery wall based on the data from the present studies; endothelial cells; smooth muscle cells; macrophages; LDL; LpL; CSPG; HSPG; DSPG.

(14) and LpL (29) interact with PG via the GAG chains, and GAG-LDL interaction involves LDL apoB (14). In addition, PG core protein-LDL interaction may exist for DSPG. The interaction between LDL and LpL is poorly understood but may involve LDL phospholipids rather than protein (7). Thus, LpL may provide additional PG-LDL binding capacity via a phospholipid-linked bridge mechanism. At low PG levels, LpL enhances binding of both CSPG and DSPG to LDL by providing additional binding sites for the PG. At higher levels of CSPG, the large molecular size of the PG may mask these additional binding sites. For the smaller sized DSPG, increased binding in the presence of LpL is observed over a wide range of concentrations.

The results of these studies suggest a hypothetical mechanism for processes leading to lipid accumulation in the artery wall (**Fig. 9**). In normal artery (Fig. 9a), plasma LDL that crosses the endothelium is transported through the arterial tissue with only limited retention by arterial wall PG. In atherosclerotic artery (Fig. 9b), the presence of macrophages modifies smooth muscle cell metabolism to produce increased DSPG (43). LpL produced by both smooth muscle cells and macrophages (2) binds to both CSPG and DSPG. This increases the interaction of these PG with LDL, thus leading to enhanced LDL entrapment in the tissue. LDL, either in a complex with PG-LpL, or following modification by its interaction with PG-LpL, is taken up by macrophages leading to foam cell formation.

The authors thank Mary Jo Wright and Abraham K. Gebre for excellent technical assistance, and Mary Jo Wright and Lonnie Ellis for assistance with preparation of the manuscript. This work has been supported in part by NIH grants HL-45848, HL-25161, and HL-45095.

*Manuscript received 8 September 1992 and in revised form 31 December 1992.*

## REFERENCES


1. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60:** 473–485.
2. Ylä-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witztum. 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* **88:** 10143–10147.
3. O'Brien, K. D., D. Gordon, S. Deeb, M. Ferguson, and A. Chait. 1992. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* **89:** 1544–1550.
4. Saxena, U., M. G. Klein, T. M. Vanni, and I. J. Goldberg. 1992. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. *J. Clin. Invest.* **89:** 373–380.
5. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 1992. Heparan sulphate proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. *Biochem. Biophys. Res. Commun.* **185:** 582–587.
6. Williams, K. J., G. M. Fless, K. A. Perie, M. L. Snyder, R. W. Brocia, and T. L. Swenson. 1992. Mechanisms by which lipolipoprotein lipase alters cellular metabolism of lipoprotein[a], low density lipoprotein, and nascent lipoproteins. *J. Biol. Chem.* **267:** 13284–13292.
7. Rumsey, S. C., J. C. Obunike, Y. Arad, R. Deckelbaum, and I. J. Goldberg. 1992. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J. Clin. Invest.* **90:** 1504–1512.
8. Schwenke, D. C., and T. E. Carew. 1989. Initiation of atherosclerotic lesion in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis.* **9:** 908–918.
9. Haberland, M. E., D. Fong, and L. Cheng. 1988. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science.* **241:** 215–218.
10. Ylä-Herttuala, S., W. Palinski, M. E. Rosenfeld, S. Parthasarathy, T. E. Carew, S. Butler, J. L. Witztum, and D. Steinberg. 1989. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.* **84:** 1086–1095.
11. Hoff, H. F., W. A. Bradley, C. L. Heideman, J. W. Gaubatz, M. D. Karagas, and A. M. Gotto, Jr. 1979. Characterization of low density lipoprotein-like particles in the human aorta from grossly normal and atherosclerotic regions. *Biochim. Biophys. Acta.* **573:** 361–374.
12. Hollander, W., J. Paddock, and M. Colombo. 1979. Lipoproteins in human atherosclerotic vessels. I. Biochemical properties of arterial low density lipoproteins. *Exp. Mol. Pathol.* **30:** 144–171.
13. Wagner, W. D. 1985. Proteoglycan structure and function as related to atherosclerosis. *Ann. N.Y. Acad. Sci.* **454:** 52–68.
14. Iverius, P-H. 1972. The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. *J. Biol. Chem.* **247:** 2607–2613.
15. Salisbury, B. G. J., D. J. Falcone, and C. R. Minick. 1985. Insoluble low-density lipoprotein-proteoglycan complexes enhance cholesteryl ester accumulation in macrophages. *Am. J. Pathol.* **120:** 6–11.
16. Vijayagopal, P., S. R. Srinivasan, K. M. Jones, B. Radhakrishnamurthy, and G. S. Berenson. 1985. Complexes of low-density lipoproteins and arterial proteoglycan aggregates promote cholesteryl ester accumulation in mouse macrophages. *Biochim. Biophys. Acta.* **837:** 251–261.
17. Hurt, E., and G. Camejo. 1987. Effect of arterial proteoglycans on the interaction of LDL with human monocyte-derived macrophages. *Atherosclerosis.* **67:** 115–126.
18. Edwards, I. J., A. K. Gebre, W. D. Wagner, and J. S. Parks. 1991. Reduced proteoglycan binding of low density lipoproteins from monkeys (*Macaca fascicularis*). *Arterioscler. Thromb.* **11:** 1778–1785.
19. Parks, J. S., and A. K. Gebre. 1991. Studies on the effect of dietary fish oil on the physical and chemical properties of low density lipoproteins in cynomolgus monkeys. *J. Lipid Res.* **32:** 305–315.
20. Salisbury, B. G. J., and W. D. Wagner. 1981. Isolation and preliminary characterization of proteoglycans dissociatively extracted from human aorta. *J. Biol. Chem.* **256:** 8050–8057.
21. Wagner, W. D., B. G. J. Salisbury, and H. A. Rowe. 1986. A proposed structure of chondroitin 6-sulfate proteoglycan of human normal and adjacent atherosclerotic plaque. *Arteriosclerosis.* **6:** 407–417.
22. Steele, R. H., W. D. Wagner, H. A. Rowe, and I. J. Edwards. 1987. Artery wall-derived proteoglycan-plasma lipoprotein interaction: lipoprotein binding properties of extracted proteoglycans. *Atherosclerosis.* **65:** 51–62.
23. Blumenkrantz, N., and G. Asboe-Hansen. 1973. New method for the determination of uronic acids. *Anal. Biochem.* **54:** 484–489.
24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
25. Christner, J. E., and J. R. Baker. 1990. A competitive assay of lipoprotein: proteoglycan interaction using a 96-well microtitration plate. *Anal. Biochem.* **184:** 388–394.
26. Socorro, L., C. C. Green, and R. L. Jackson. 1985. Preparation of a homogenous and stable form of bovine milk lipoprotein lipase. *Prep. Biochem.* **15:** 133–143.
27. Saxena, U., L. D. Witte, and I. J. Goldberg. 1989. Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *J. Biol. Chem.* **264:** 4349–4355.
28. Koritnik, D. L., and L. L. Rudel. 1983. Measurement of apolipoprotein A-I concentration in nonhuman primate serum by enzyme-linked immunosorbent assay (ELISA). *J. Lipid Res.* **24:** 1639–1645.
29. Margelin, D., A-M. Pigier, J. Gardette, J. Bertrand, N. Vu-Dac, and J. Picard. 1989. Evidence for an interaction of lipoprotein lipase with artery wall proteoglycans. *Int. J. Biochem.* **21:** 761–768.
30. Fransson, L-A., and B. G. Johansson. 1981. Glycosaminoglycan-lipoprotein interactions. 1. Effect of uronic acid composition and charge density of the glycan. *Int. J. Biol. Macromol.* **3:** 25–30.
31. Fransson, L-A., and B. Havsmark. 1982. Glycosaminoglycan-lipoprotein interactions. 3. Structure of dermatan sulphate variants with high affinity for low density lipoprotein. *Biol. Macromol.* **4:** 50–54.
32. Stevens, R. L., M. Colombo, J. J. Gonzales, W. Hollander, and K. Schmid. 1976. The glycosaminoglycans of the human artery and their changes in atherosclerosis. *J. Clin. Invest.* **58:** 470–481.
33. Tammi, M., P. O. Seppälä, A. Lehtonen, and M. Möttönen. 1978. Connective tissue components in normal and atherosclerotic human coronary arteries. *Atherosclerosis.* **29:** 191–194.
34. Wagner, W. D., and B. G. J. Salisbury. 1978. Aortic total

glycosaminoglycan and dermatan sulfate changes in atherosclerotic rhesus monkeys. *Lab. Invest.* **39:** 322–328.
35. Wagner, W. D., and S. R. Nohlgren. 1981. Aortic glycosaminoglycans in genetically selected WC-2 pigeons with increased atherosclerosis susceptibility. *Arteriosclerosis.* **1:** 192–201.
36. Kumar, V., G. S. Berenson, H. Ruiz, E. R. Dalferes, Jr., and J. P. Strong. 1967. Acid mucopolysaccharides of human aorta. *J. Atheroscler. Res.* **7:** 583–590.
37. Hollmann, J., A. Schmidt, D-B. von Bassewitz, and E. Buddecke. 1989. Relationship of sulfated glycosaminoglycans and cholesterol content in normal and arteriosclerotic human aorta. *Arteriosclerosis.* **9:** 154–158.
38. Saxena, U., M. G. Klein, and I. J. Goldberg. 1991. Identification and characterization of the endothelial cell surface lipoprotein lipase receptor. *J. Biol. Chem.* **266:** 17516–17521.
39. Gallagher, J. T., M. Lyon, and W. P. Steward. 1986. Structure and function of heparan sulphate proteoglycans. *Biochem. J.* **236:** 313–325.
40. Bengtsson, G., T. Olivecrona, M. Hook, J. Riesenfeld, and U. Lindahl. 1980. Interaction of lipoprotein lipase with native and modified heparin-like polysaccharides. *Biochem. J.* **189:** 625–633.
41. Aviram, M., E. L. Bierman, and A. Chait. 1988. Modification of low density lipoprotein by lipoprotein lipase or hepatic lipase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **263:** 15416–15422.
42. Camejo, G., S-O. Olofsson, F. Lopez, P. Carlsson, and G. Bondjers. 1988. Identification of apoB-100 segments mediating the interaction of low density lipoproteins with arterial proteoglycans. *Arteriosclerosis.* **8:** 368–377.
43. Edwards, I. J., W. D. Wagner, and R. T. Owens. 1990. Macrophage secretory products selectively stimulate dermatan sulfate proteoglycan production in cultured arterial smooth muscle cells. *Am. J. Pathol.* **136:** 609–621.