Lipoprotein Lipase Increases Low Density Lipoprotein Retention by Subendothelial Cell Matrix

Uday Saxena, Michael G. Klein, Teresa M. Vanni, and Ira J. Goldberg
Department of Medicine and Specialized Center of Research in Arteriosclerosis, Columbia University, College of Physicians and Surgeons, New York 10032

Abstract

Lipoprotein lipase (LPL), the rate-limiting enzyme for hydrolysis of plasma lipoprotein triglycerides, is a normal constituent of the arterial wall. We explored whether LPL affects (*a*) lipoprotein transport across bovine aortic endothelial cells or (*b*) lipoprotein binding to subendothelial cell matrix (retention). When bovine milk LPL was added to endothelial cell monolayers before addition of ^{125}I-labeled LDL, LDL transport across the monolayers was unchanged; but, at all concentrations of LDL tested (1–100 μg), LDL retention by the monolayers increased more than fourfold. ^{125}I-labeled LDL binding to extracellular matrix increased when LPL was added directly to the matrix or was added to the basolateral side of the endothelial cell monolayers. Increased LDL binding required the presence of LPL and was not associated with LDL aggregation. LPL also increased VLDL, but not HDL, retention. Monoclonal anti-LPL IgG decreased both VLDL and LDL retention in the presence of LPL. Lipoprotein transport across the monolayers increased during hydrolysis of VLDL triglyceride (TG). In the presence of LPL and VLDL, VLDL transport across the monolayers increased 18% and LDL transport increased 37%. High molar concentrations of oleic acid to bovine serum albumin (3:1) in the medium increased VLDL transport ~ 30%. LDL transport increased 42% when oleic acid was added to the media. Therefore, LPL primarily increased retention of LDL and VLDL. A less remarkable increase in lipoprotein transport was found during hydrolysis of TG-containing lipoproteins. We hypothesize that LPL-mediated VLDL and LDL retention within the arterial wall potentiates conversion of these lipoproteins to more atherogenic forms. (*J. Clin. Invest.* 1992. 89:373–380.) Key words: atherosclerosis • endothelial cells • very low density lipoproteins • proteoglycans • heparan sulfate

Introduction

Lipoprotein lipase (LPL),[1] the principal enzyme responsible for hydrolysis of lipoprotein triglyceride (TG), is synthesized primarily in adipocytes and myocytes. LPL is then released into the interstitial space, crosses the endothelial cell barrier, and attaches to heparan sulfate proteoglycans on the luminal surface of capillary endothelium. Intravenous injection of heparin into humans or animals dissociates LPL from this binding site, resulting in LPL activity in postheparin plasma. LPL initiates chylomicron metabolism (1) and conversion of VLDL to LDL (2). LPL actions, at least in part, regulate HDL levels (3). LPL activity in postheparin plasma is positively correlated with HDL cholesterol levels in humans (4). Because HDL levels are inversely related to risk for coronary artery disease, these data suggest that LPL actions on circulating lipoproteins protect against the development of atherosclerosis.

The artery wall has a small amount of LPL activity that is unlikely to be important in plasma lipoprotein regulation. LPL activity in rabbit aortas correlates with the cholesterol content of the arteries (5). This relationship, in part, led Zilversmit (5) to hypothesize that arterial wall LPL promotes atherosclerosis. The site of arterial LPL synthesis was recently demonstrated. LPL mRNA is in arterial macrophage-derived foam cells (6, 7); LPL protein is in similar areas (7). Some LPL is also found in areas containing smooth muscle cells (7, 8). LPL is, therefore, a constituent of the atherosclerotic plaque. The physiological or pathophysiological role of LPL within the vessel wall is unknown.

A number of steps are required for the pathological effects of LDL on the arterial wall. These include LDL permeation of the endothelial cell barrier, LDL retention in the arterial wall, and LDL modification to more atherogenic forms (9, 10). Increases in the amounts of LDL involved in any of these steps will, theoretically, increase the rate of formation of atherosclerotic plaques. Some circulating LDL normally cross the endothelial cell barrier (11). Most of this LDL travels through the vessel wall and reenters the circulation via the lymphatics (12). LDL, however, is found in atherosclerotic lesions (13), suggesting that during atherogenesis LDL are trapped in the subendothelial space. Moreover, atherosclerosis-sensitive areas of rabbit arteries retain more plasma LDL than adjacent atherosclerosis-resistant areas (14). Thus LDL retention may be a primary event in the atherogenic process.

As a model for the arterial intima, we have studied LPL binding to cultured endothelial cells. Because endothelial cells do not synthesize LPL, approximation of the physiological situation required addition of purified LPL to the cultured cells. Using this system, we have explored factors that release LPL from endothelial cell apical surfaces (15) and mediate LPL transport across endothelial cells grown on semipermeable filters (16). In the current experiments, we studied the effects of LPL on lipoprotein transport across monolayers of cultured endothelial cells. Our data show that LPL markedly increases LDL and VLDL association with subendothelial cell matrix. These findings suggest a mechanism for increased LDL retention in atherosclerotic lesions.

Address correspondence to Dr. Ira J. Goldberg, Department of Medicine, Columbia University, 630 West 168 Street, New York, NY 10032. Dr. Saxena's current address is Parke-Davis Pharmaceutical Research Division, 2800 Plymouth Road, Ann Arbor, MI 48105.

Received for publication 3 July 1991 and in revised form 3 October 1991.

1. *Abbreviations used in this paper:* LPL, lipoprotein lipase; TG, triglyceride.

J. Clin. Invest.

0021-9738/92/02/0373/08 $2.00
Volume 89, February 1992, 373–380

Methods

Isolation of lipoproteins and bovine milk LPL. VLDL, LDL, and HDL were isolated from human plasma by sequential isopynic ultracentrifugation (17). TG and cholesterol measurements were performed using an automated enzymatic analyzer (model ABA 100; Abbott Laboratories, North Chicago, IL). VLDL, LDL, and HDL were radioiodinated using iodine monochloride (18). ^{125}I and ^{131}I not associated with protein were removed by gel filtration using a PD-10 column (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) followed by dialysis against 150 mM NaCl, 10 mM sodium phosphate, pH 7.4 (PBS). Radioactivity was measured using a gamma counter (model 1274; LKB Instruments, Gaithersburg, MD). Over 90% of VLDL, LDL, and HDL radioactivity was precipitated using 10% TCA. ^{125}I-labeled LDL specific radioactivity was ~ 16,000 cpm/μg; the specific radioactivity of ^{131}I-labeled VLDL was 8,500 cpm/μg; ^{125}I-labeled HDL specific radioactivity was 226,000 cpm/μg.

LPL was purified from fresh unpasteurized bovine milk using heparin-agarose (Bio-Rad Laboratories, Richmond, CA) affinity chromatography and the specific activity determined as described previously (15). At the time of storage, LPL preparations had a specific activity of ~ 20–30 mmol FFA/h per mg of protein. The purified enzyme was stored at −70°C.

Endothelial cell monolayers. Primary cultures of bovine aortic endothelial cells were established as previously described for porcine aortic endothelial cells (15). At confluence, the endothelial cells were subcultured using 0.125% trypsin (Gibco Laboratories, Grand Island, NY) and 0.02% EDTA. Cells were plated onto 25-mm polycarbonate filters (3.0-μm pore diameter, Nuclepore, Pleasanton, CA), according to the method of Shasby and Shasby (19) as described previously (16). The media in the upper chambers (1.5 ml) and lower chambers (2.6 ml), separated by the filter, were replaced every other day. Experiments were conducted 5–6 d after seeding the endothelial cells.

The barrier function of the endothelial cell monolayer was examined as previously reported (16), using [^{3}H]dextran (average mol wt 150,000) and [^{14}C]albumin. Transport of these molecules from the apical to the basolateral side of the monolayers was < 1.5%/h, a rate similar to that reported by others (20). At the conclusion of each LDL transport experiment, the monolayers were stained with 2% toluidene blue to verify the uniformity of the monolayer.

Transport studies. On the day of the experiment, culture media from both chambers were aspirated and the cells were washed three times with DME containing 3% BSA (DME-BSA). LPL was associated with the endothelial cell monolayers by adding purified LPL to the upper-chamber medium, incubating the cells for 45 min at 37°C, and washed with DMEM-BSA to remove unbound LPL. In other experiments, LPL was added to medium in the lower chamber to allow LPL to associate preferentially with the subendothelial cell matrix and with the basolateral surface of the endothelial cells.

After addition of ^{125}I-labeled LDL, ^{125}I-labeled HDL, or ^{131}I-labeled VLDL to the upper chamber, radioactivity in the lower-chamber medium and associated with the extracellular matrix was determined. The total amount of each lipoprotein transported across the monolayers (net transport) is the sum of these two measurements. To determine ^{125}I-labeled LDL associated with the subendothelial cell matrix, the cells were washed three times with cold DME-BSA. DME containing 50 U/ml of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) was then added to the lower chamber and the radioactivity released from the matrix was quantified after a 10-min, 4°C incubation. In some experiments, before the heparin treatment the endothelial cell layer was removed from the filters to examine the lipoproteins specifically associated with the subendothelial matrix.

Results

^{125}I-labeled LDL transport across and retention by endothelial cell monolayers. To determine whether LPL on endothelial cells alters the amount of LDL transport across or retention by the monolayers, ^{125}I-labeled LDL (1–100 μg/ml) were added to chambers above control and LPL-containing monolayers. When greater concentrations of LDL were included in the upper-chamber medium, more ^{125}I-labeled LDL was found in the lower chamber after the 1-h incubation. Preincubation with LPL (10 μg/ml) did not alter LDL transport across the monolayers (Fig. 1 *A*). But compared with control, LPL-treated monolayers had more ^{125}I-labeled LDL released from the basolateral side of the cells by heparin (Fig. 1 *B*). Under control conditions, 1–15 ng of LDL was released with heparin; when LPL was present, 5.8–64.5 ng of LDL was retained by the monolayers. When LDL concentrations were increased, the percentage of transported LDL retained by LPL-treated monolayers decreased from 10 to 2.2%. This suggested that LDL binding to the LPL was approaching saturation. Nonetheless, at all concentrations of LDL, LPL increased LDL retention more than fourfold.

An experiment was conducted to test whether LPL would increase LDL retention in the presence of serum. Tracer ^{125}I-la-

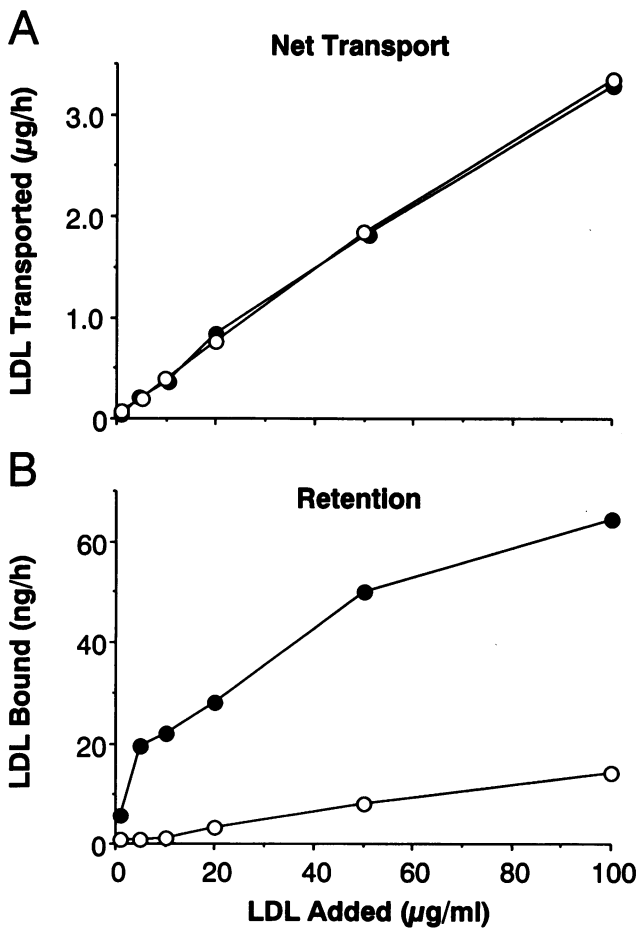

Figure 1. LDL transport across and retention by endothelial cell monolayers: effect of LPL addition. The transport of ^{125}I-labeled LDL (1–100 μg) across confluent monolayers of bovine endothelial cells was studied using control and LPL (10 μg)-treated monolayers. Shown are the amounts of LDL transported (*A*) and retained by the monolayers (*Retention, B*) at each dosage. Under control conditions, < 0.5% of the total transported LDL was released from the basolateral side of the monolayers. Results shown are means of experiments performed in triplicate. ●, +LPL; ○, control.

beled LDL in 1 ml of human serum (cholesterol 170 mg/dl) and 0.5 ml of DME-BSA were added to the upper chambers of control and LPL-treated endothelial cell monolayers. After 1 h at 37°C, under control conditions (no LPL), < 1% of the transported LDL was retained by the matrix. In the presence of LPL, eight times more ^{125}I-labeled LDL was retained (data not shown). Thus, even in the presence of serum and physiological concentrations of LDL, LPL marked increased LDL retention.

LDL association with extracellular matrix. LDL released from the basolateral side of endothelial cell monolayers by heparin could include ^{125}I-labeled LDL associated with the filter, the basolateral surface of the cells, and the matrix. We therefore assessed whether LPL specifically increased LDL binding to subendothelial matrix. Confluent monolayers of cultured endothelial cells were incubated in 3 mM EDTA in PBS for 1 h at room temperature. The cells were then removed with a rubber policeman, leaving the subendothelial cell matrix on the filter. LPL (10 μg) was allowed to associate with the matrix for 45 min at 37°C. ^{125}I-labeled LDL (3 μg) was then incubated for 1 h at 37°C with control filters and filters containing LPL. ^{125}I-labeled LDL bound to the matrix was released with heparin-containing buffer. As shown in Figure 2 *A*, 1.7 ng of the added LDL bound to the control matrix, whereas 201 ng of LDL bound to the LPL-treated matrix. A similar experiment was performed using gelatin-fibronectin–treated filters not used for growth of endothelial cells. Therefore, these filters did not contain endothelial cell–derived matrix. LPL-treated filters containing no matrix retained < 1% of the LDL found on LPL-treated filters containing subendothelial cell matrix. The increase in LDL released by heparin from the basolateral side of endothelial cell monolayers thus was due, at least in part, to increased LPL-mediated LDL association with the matrix.

LDL binding to subendothelial matrix of control and LPL-treated monolayers was assessed. ^{125}I-labeled LDL (3 μg/ml) in DME-BSA was added to the media on the apical side of the cells. After 1 h at 37°C, the cells were washed and removed from the filters using a rubber policeman. In LPL-treated monolayers, 3.4 ± 0.6 ng (mean±SD) of LDL was released by heparin from the residual filter-associated matrix; 0.99±0.05 ng of LDL was released from control monolayers. It should be noted that during removal of the endothelial cells it is likely that some of the matrix was also removed from the cells. Nonetheless, LPL treatment caused a more than threefold increase in matrix-associated LDL.

LPL addition to the subendothelial space. After cultured monolayers are incubated with LPL, LPL is bound to endothelial cell surfaces and extracellular matrix and is internalized by the cells. In the experiments shown in Fig. 1, LPL was added to the cells by its inclusion in the upper-chamber medium. Because LPL is synthesized by arterial cells beneath the endothelial layer, a more physiological situation is to add the LPL to the lower, rather than the upper, chamber medium. The data shown in Fig. 2 *B* were obtained from experiments in which increasing concentrations of LPL were added to the lower chamber. No changes in net LDL transport from the upper to lower chambers were found. But addition of more LPL increased the proportion of transported ^{125}I-labeled LDL bound to the matrix while the amount of LDL in the media decreased from 75 to 50 ng. LDL retention appeared to reach saturation. With addition of 100 or 300 μg of LPL, ~ 30 ng of LDL was retained by the monolayers. It should be noted that much greater amounts of LPL were used in these experiments than in the experiments shown in Fig. 1 and the greater amounts of LPL increased total LDL retention. Therefore, LDL retention in Figure 1 was limited by the amount of LPL associated with the monolayers.

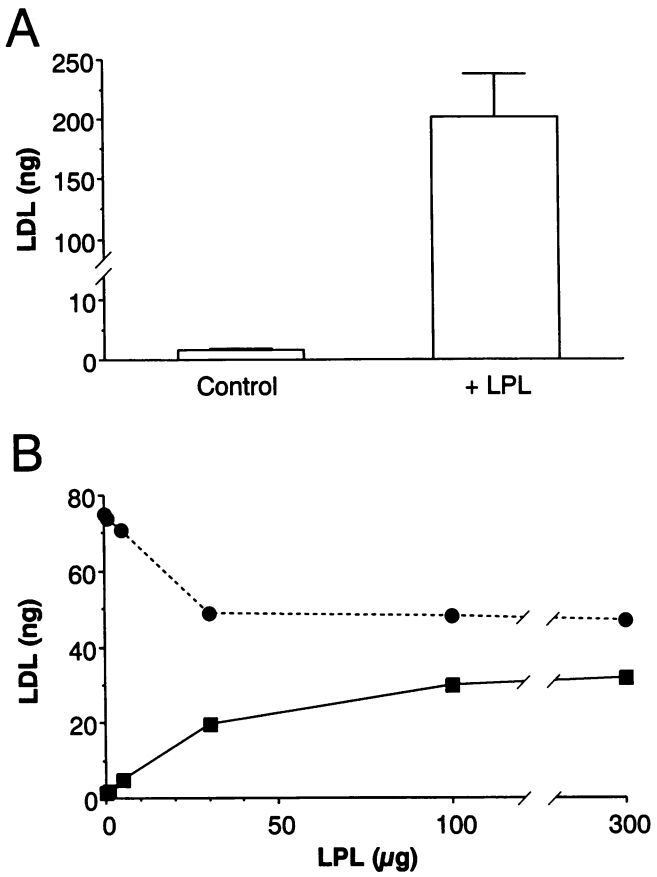

Figure 2. Association of LDL with extracellular matrix. (*A*) Direct binding of ^{125}I-labeled LDL to extracellular matrix. Bovine aortic endothelial cells were grown to confluence on filters. The cells were detached with EDTA as described in Methods. The binding of LDL (3 μg) to the matrix in the presence and absence of LPL (prebound to the matrix) was determined. (*B*) Retention of LDL after addition of LPL to the basolateral endothelial cell surface. LPL was added to the lower chamber–basolateral surface of endothelial cells, and the cells were incubated for 45 min at 37°C. After removal of the unbound LPL, 3 μg of ^{125}I-labeled LDL was added to the upper chamber media, and the amounts of LDL in the lower-chamber media and retained by the matrix were determined. Data shown are the means of triplicate experiments. ●, media; ■, matrix.

Mechanism of increased LDL retention. LDL association with the matrix could increase by two mechanisms. (*a*) LDL aggregated or altered in some other manner by the enzymatic actions of LPL may have a greater affinity for proteoglycans. (*b*) Macromolecular complexes of LPL and LDL might form and bind more avidly to the matrix. To assess whether LPL had altered the size of the LDL particles, for example, by causing aggregation, LDL (12 μg) was incubated for 1 h at 37°C with increasing quantities of LPL (0–16 μg). As shown in Fig. 3 *A*, LPL-treated LDL was analyzed by PAGE using 2–16% nondenaturing gels that were stained with Sudan black (21). Even with these relatively large amounts of LPL, LDL aggregation was not observed. In some experiments, LPL treatment caused a slight decrease in LDL size. In another experiment, ^{125}I-la-

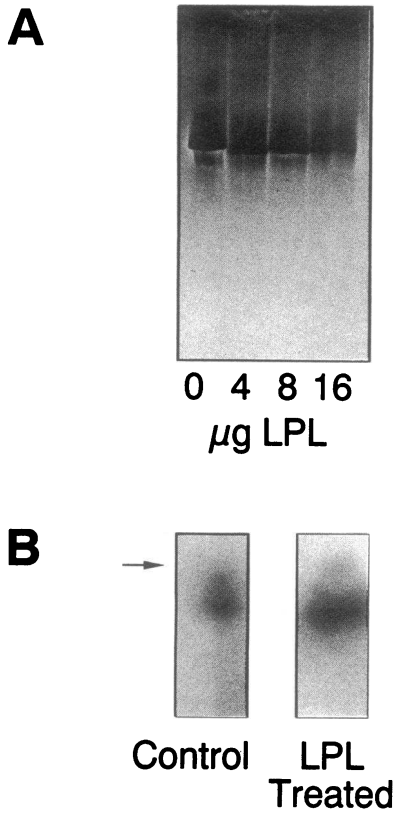

Figure 3. Nondenaturing gel electrophoresis of LPL-treated LDL. (*A*) LDL (12 μg) were incubated for 1 h at 37°C with 0, 4, 8, or 16 μg of LPL. The LDL preparations were then analyzed by nondenaturing gel electrophoresis using 2–16% gradient gels that were stained with Sudan black. (*B*) ^{125}I-labeled LDL and ^{125}I-labeled LDL heparin released from LPL-treated subendothelial matrix were analyzed by gel electrophoresis as described in *A*. Autoradiography was performed using Kodak X-omat AR Film. Shown is a 24-h exposure of control LDL (*left lane*) and matrix LDL (*right lane*). The top of the gel is indicated by the arrow.

beled LDL bound to LPL-treated subendothelial matrix was assessed by gel electrophoresis and autoradiography. As shown in Figure 3 *B*, ^{125}I-labeled LDL released from the matrix by heparin was similar in size and amount of aggregated material to control LDL. Thus, increased LDL binding was not due to LDL aggregation.

The role of LPL protein in ^{125}I-labeled LDL binding to heparin was also evaluated. 200 μg of ^{125}I-labeled LDL mixed with 20 μg of LPL was incubated for 1 h at 37°C. The mixture was divided, and half the LDL was reisolated by ultracentrifugation followed by dialysis against PBS. We have previously reported that ultracentrifugation will dissociate most LPL from LDL (22). Control ^{125}I-labeled LDL, ^{125}I-labeled LDL mixed with LPL, and ^{125}I-labeled LDL mixed with LPL and reisolated by ultracentrifugation were added to 200 μl of PBS–3% BSA and then incubated with 200 μl of heparin–Sepharose gel (Pharmacia) for 1 h at 37°C. After the gel was washed with PBS-BSA, 5% of the added ^{125}I-labeled LDL was associated with the gel under control conditions, whereas 18% of the LPL-LDL was associated with the gel. LPL–^{125}I-labeled LDL reisolated by ultracentrifugation (conditions that should dissociate LPL from the LDL) bound to heparin–Sepharose gel at control levels, ~ 4%. Experiments using ^{125}I-labeled LPL and unlabeled LDL confirmed that during the ultracentrifugation > 85% of the labeled LPL was dissociated from LDL and recovered in the $d > 1.063$ g/ml density fraction. Therefore, the presence of LPL, not alterations in LDL structure, probably increased LDL retention by glycosaminoglycans on heparin–Sepharose and on the extracellular matrix.

^{125}I-labeled LDL transport and retention in the presence of VLDL. The effects of VLDL lipolysis on LDL retention by endothelial cell monolayers were explored in a double-label experiment using ^{125}I-labeled LDL and ^{131}I-labeled VLDL. VLDL was obtained from a mildly hypertriglyceridemic subject (plasma TG = 223 mg/dl). Monolayers were incubated for 1 h at 37°C with media containing LDL (15 μg) and VLDL (204 μg). In these experiments we used monolayers with (*a*) no LPL (control), (*b*) associated LPL (bound LPL), (*c*) prebound LPL and 40 μg of anti–LPL MAb in the medium, and (*d*) LPL included in the lipoprotein-containing medium (denoted LPL in solution in Figs. 4 and 5). Compared with control, association of LPL with the monolayers increased net ^{125}I-labeled LDL transport > 35% (Fig. 4 *A*). Prebound LPL had no effect on transport of [^{3}H]dextran across the monolayers. This in-

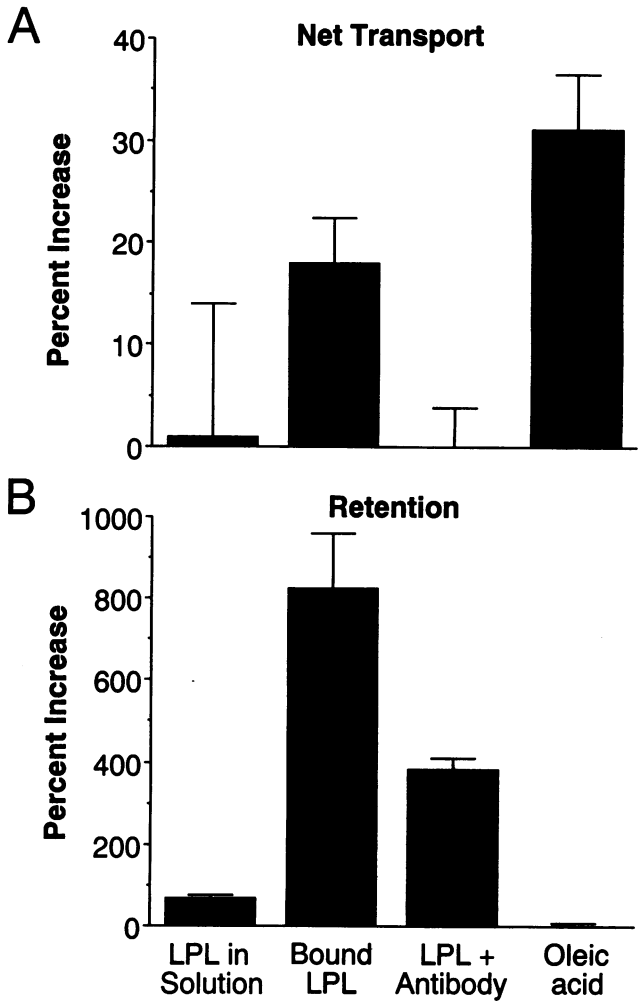

Figure 4. Transport of LDL in the presence of VLDL. (*A*) Net transport of ^{125}I-labeled LDL. The transport of iodinated human LDL (15 μg) across confluent monolayers of bovine endothelial cells was studied in the presence of VLDL (204 μg). Control LDL transport was compared with transport in the presence of LPL (250 μg) to the medium (LPL in solution), after preincubation of cells with LPL (bound LPL), after preincubation with LPL and addition of anti–LPL antibodies to the medium, and with addition of medium containing a 3:1 molar ratio of oleic acid to 3% bovine serum albumin. Results shown are mean±SEM of experiments performed in triplicate, as the percent increase compared with control values. (*B*). Retention of LDL by extracellular matrix. The amounts of iodinated human LDL (15 μg) retained by subendothelial cell extracellular matrix were determined under the conditions described in *A*. Results shown are mean±SEM of experiments performed in triplicate.

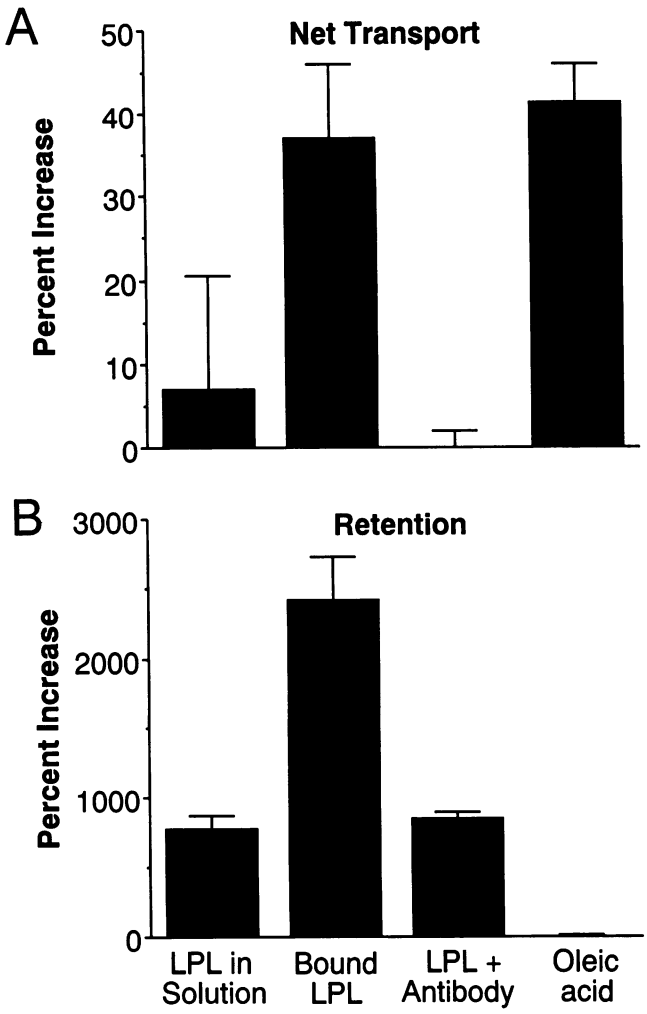

Figure 5. Transport and retention of VLDL. (*A*) Net transport of VLDL. The transport of ^{131}I-labeled VLDL (204 μg) across confluent monolayers of bovine aortic endothelial cells was studied under control conditions, after preincubation of the cells with LPL (250 μg) (bound LPL), with addition of LPL to the VLDL-containing medium (LPL in solution), after preincubation with LPL and addition of anti-LPL antibodies, and in medium containing a 3:1 molar ratio of oleic acid to 3% bovine serum albumin. Results shown are mean±SEM of experiments performed in triplicate, as the percent increase compared with control values. (*B*) Retention of VLDL by monolayers. The amounts of iodinated human VLDL (204 μg) retained by endothelial cell monolayers were determined under the conditions described in *A*. Results shown are mean±SEM of experiments performed in triplicate.

crease in LDL net transport using prebound LPL was blocked by anti-LPL antibodies (Fig. 4 *A*).

FFA or other products of VLDL lipolysis may have increased LDL transport. Both LPL on the surface of endothelial cells and LPL in solution hydrolyze VLDL TG, but endothelial cells are exposed to higher local concentrations of lipolysis products when LPL is on the cell surface. To test whether LPL in solution would be equally effective in increasing LDL transport, LPL was added to VLDL containing medium. LPL in solution did not increase LDL transport (Fig. 4 *A*).

To assess whether LDL transport was altered by FFA alone, lipoprotein-containing medium with a 3:1 ratio of oleic acid/BSA was added to the apical side of the cells. Addition of oleic acid increased LDL transport 41.5% (Fig. 4 *A, right bar*). Transport of dextran did not increase during these 1-h experiments, suggesting that short-term incubation with oleic acid did not alter the integrity of the monolayer. Therefore, oleic acid and perhaps other VLDL lipolysis products increased LDL transport without damaging endothelial cell monolayers.

A marked increase in ^{125}I-labeled LDL retention by the monolayers was found in the presence of LPL. In this experiment, LDL retention increased approximately 8-fold if the LPL was in solution (Fig. 4 *B, left bar*) and 24-fold if the LPL was first allowed to bind to the cells (Fig. 4 *B*). If LPL was first allowed to bind to the cells and then anti-LPL IgG added, the retention of ^{125}I-labeled LDL by the matrix was 62% less (Fig. 4 *B, right bar*). Oleic acid did not increase LDL retention. Thus, when LPL was active and/or able to interact with lipoproteins it increased LDL retention. It is likely that prebinding LPL allowed more LPL to associate with the matrix, increasing the amount of LPL available to bind LDL.

VLDL transport and retention. VLDL transport across monolayers of cultured endothelial cells was also assessed in the presence and absence of LPL. If LPL was allowed to bind to the cells and VLDL was added, a small (18%) increase in VLDL net transport across the monolayers was found (Fig. 5 *A*). No net increase in VLDL transport was observed if the LPL and VLDL were mixed before their addition to the upper chamber. Because lipolysis of VLDL produces smaller, remnant lipoproteins, the increase in transport could have resulted from the formation of smaller lipoproteins. However, addition of medium containing a 3:1 molar ratio of oleic acid/BSA also increased VLDL transport by 30%. Thus, even without lipolysis and the generation of smaller lipoproteins, oleic acid increased VLDL transport.

VLDL retention increased when LPL was added to the monolayers. Compared with control conditions, LPL addition produced an eightfold increase in monolayer-associated VLDL (Fig. 5 *B*). This increase was most marked when LPL was first bound to the cells rather than added to the cells along with the lipoproteins. Addition of anti-LPL IgG to endothelial cells containing prebound LPL led to a 50% decrease in VLDL retention by the matrix. Although high molar ratios of oleic acid/BSA increased VLDL transport, they did not increase VLDL retention. Thus, VLDL retention by the monolayers required LPL and was blocked by an antibody that inhibits VLDL interaction with LPL (23).

Comparison of ^{125}I-labeled LDL and ^{125}I-labeled HDL. Transport and retention of HDL and LDL were compared. In parallel experiments, tracer quantities of ^{125}I-labeled LDL and ^{125}I-labeled HDL in DME containing 10% normolipidemic human serum were added to the apical side of control and LPL-treated (10 μg/ml) endothelial cell monolayers. At the end of the 1-h incubation, 2.8% of the LDL and 1.4% of the HDL radioactivity was found in the lower chamber. LPL treatment did not alter the amounts of either LDL or HDL tracers transported to the lower chamber. Serum contains more HDL than LDL molecules; therefore, despite the lower percent HDL than LDL radioactivity in the lower chamber, more HDL than LDL was probably transported during the 1-h incubation.

LPL treatment of the monolayers affected only LDL retention. As shown in Figure 6, LPL treatment caused a more than fourfold increase in retained LDL. LPL treatment also increased the amounts of LPL associated with the endothelial

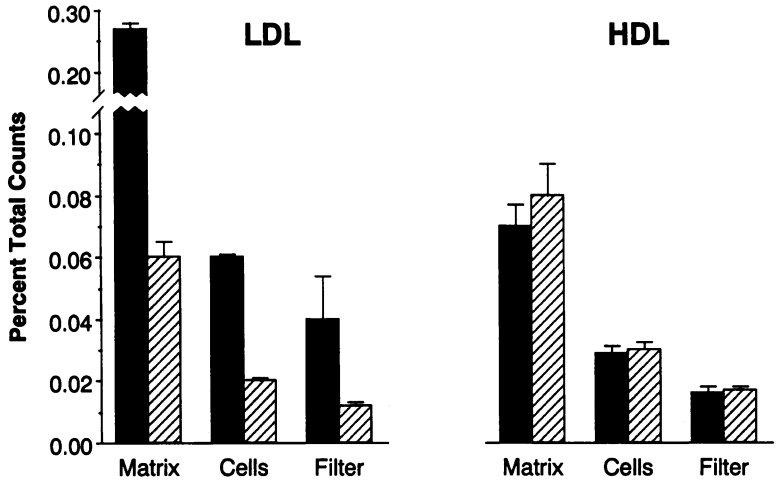

Figure 6. Comparison of ^{125}I-labeled LDL and ^{125}I-labeled HDL. ^{125}I-labeled LDL (634,000 cpm) or ^{125}I-labeled HDL (333,000 cpm) were added to media (DME with 10% human serum) on the apical side of control and LPL-treated (10 μg/ml) monolayers. After a 1-h incubation at 37°C, amounts of radioactivity released from the monolayers by heparin (denoted matrix), associated with the scraped cells, and remaining with the filters were determined. Shown are the averages±SD of the percentage of added radioactivity from triplicate sets of monolayers. ■, LPL; ▨, control.

cells and with the filter. HDL retention was not altered by LPL addition. Therefore, LPL increased retention of VLDL and LDL, but not HDL, by the monolayers.

Discussion

Our experiments demonstrate that LPL addition increases VLDL and LDL, but not HDL, retention by cultured endothelial cell monolayers. LPL also specifically increased LDL binding to subendothelial cell matrix. The increased lipoprotein association to LPL-treated monolayers was inhibited by addition of monoclonal anti-LPL IgG. Because this antibody blocks the interaction of LPL with substrate lipoproteins (23), in these experiments the antibody probably decreased lipoprotein binding to LPL.

The mechanism responsible for LPL-mediated LDL retention was explored. One possibility is that LPL interaction with LDL leads to LDL aggregation; but, in the presence of LPL, no marked increase in LDL size assessed by gel filtration (22) and by gradient gel electrophoresis was found. Furthermore, ^{125}I-labeled LDL released from LPL-treated matrix appeared similar to control LDL. Therefore, appreciable LDL aggregation was unlikely.

A more likely possibility is that, as illustrated in Fig. 7, LPL anchors LDL molecules by binding to glycosaminoglycans and to lipoproteins. LPL has separate lipid/lipoprotein- and heparin-binding domains (24). Because LPL attached to glycosaminoglycans can hydrolyze TG (25), these two domains do not overlap. LPL binds with high affinity to heparan sulfate proteoglycans, including those on the endothelial cell surface and in the subendothelial cell matrix (26). During hydrolysis of TG-rich lipoproteins, LPL is a biochemical bridge between its substrate and proteoglycan binding site. LPL also complexes with LDL. Previous studies from this laboratory (22) and others (27) demonstrated that LPL will associate with LDL-size lipoproteins. Therefore, the biochemical conditions are appropriate for the formation of LPL-LDL complexes that also bind to matrix proteoglycans, including those within the arterial wall. This hypothesis is supported by our experimental finding that dissociation of LPL-LDL complexes by ultracentrifugation decreased LDL binding to heparin sulfate glycosaminoglycans. Thus, LDL retention required the presence of LPL, which may have increased LDL affinity for subendothelial cell matrix proteins.

Under physiological ionic conditions, LDL binds weakly, if at all, to heparin sulfate glycosaminoglycans (28). For this reason, low-ionic-strength buffers are usually employed to assess LDL interaction with heparin (29). Proteoglycans containing heparan sulfate and chondroitin sulfate glycosaminoglycans are major components of the arterial wall. Therefore, factors that promote LDL interaction with these proteoglycans may, in vivo, initiate or accelerate arterial LDL retention. LDL retention, in turn, could increase (*a*) cholesterol deposition within the vessel wall; (*b*) LDL–proteoglycan complex uptake by macrophages, leading to increased macrophage lipid (30, 31); and (*c*) LDL conversion to oxidized, more atherogenic forms (32). Oxidized LDL is a chemotactic factor for monocytes, and its uptake by macrophages results in cellular choles-

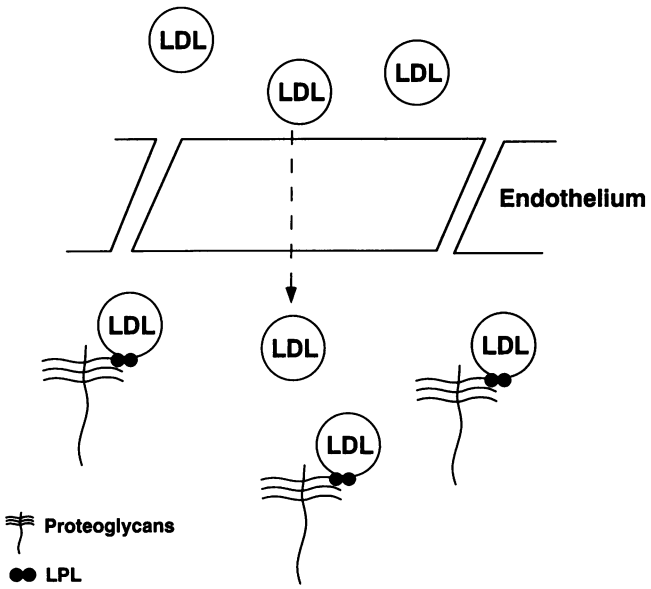

Figure 7. Proposed mechanism of LPL-mediated increased LDL retention by matrix. Small amounts of LDL in the circulation traverse the endothelial barrier. Unless retained, these LDL eventually are returned to the circulation via the lymphatics. LPL synthesized by macrophages and smooth muscle cells is attached to proteoglycans in the subendothelial matrix. Increased LDL retention in the vessel wall may occur when the lipid-binding domain of LPL interacts with LDL. During its retention within the vessel wall, LDL can then be converted to more atherogenic oxidized forms.

teryl ester enrichment (10). Therefore, we speculate that LPL-mediated LDL retention increases both lipid and cellular components of atherosclerotic lesions.

A second but less dramatic effect noted in our studies was an increase in LDL and VLDL transport across the monolayers during VLDL lipolysis and in the presence of high molar ratios of oleic acid. One reason for this increased transport may be an oleic acid–mediated increase in endothelial cell transport. Oleic acid activates protein kinase C (33), a condition which increases albumin transport across endothelial cells (34, 35). Alternatively, oleic acid or lipolysis products might have altered the integrity of the monolayers (36). Such effects occur in more chronic situations. Because dextran transport was unaltered in our acute, 1-h experiments, it suggested that the endothelial cell monolayers were intact.

Increased LDL transport was distinct from increased LDL binding to the extracellular matrix. Oleic acid increased ^{125}I-labeled LDL transport without increasing LDL retention by monolayers. Moreover, high concentrations of oleate/BSA decreased LPL-mediated LDL binding to the matrix (data not shown). Therefore, retention and transport effects occur via different mechanisms.

The extrapolation of our in vitro findings to the pathogenesis of atherosclerosis requires consideration of two important questions. Does retention of LDL correlate with areas of vessel walls that develop atherosclerosis? Schwenke and Carew (14) studied LDL permeation and retention in normal and atherosclerosis-susceptible areas in the aorta. More LDL was retained by extracellular matrix in the atherosclerosis-prone regions. In contrast, increased LDL permeability of aortic segments was not a consistent feature of atherosclerosis-sensitive sites. Do processes that increase the association of LDL with the extracellular matrix lead to potentially harmful modifications of LDL? Retained LDL may be more easily oxidized due to more prolonged exposure to oxidative enzymes. In addition, proteoglycan-bound LDL is more easily oxidized than LDL in solution (32). Oxidized LDL would then be internalized by arterial wall macrophages.

One of the pathological features of the atherosclerotic plaque is the presence of cholesteryl ester–rich cells called foam cells. Foam cells are derived from macrophages and smooth muscle cells that have increased their lipid content via uptake of lipoprotein cholesterol (37, 38). Most of this cholesterol originates in circulating LDL (10) or β-VLDL (39). Normally the amount of cholesterol within macrophages is tightly regulated. In the presence of LDL, human monocyte–derived macrophages downregulate LDL receptors, preventing cholesteryl ester enrichment of the cells (40, 41). However, LDL may be modified in the circulation or in the subendothelial cell space to forms that can, in vitro, lead to macrophages enriched in cholesteryl ester. These modifications include LDL lipid oxidation (10, 42), LDL aggregation (43, 44), and LDL association with proteoglycans in the extracellular matrix (30, 31). Although circulating monocytes do not secrete LPL, macrophages (45), including human monocyte–derived macrophages (46), synthesize LPL. We postulate that LPL-mediated LDL retention and LPL hydrolysis of TG-rich lipoproteins (47) augment conversion of macrophages to foam cells. This hypothesis is, however, based on a number of in vitro findings and requires testing in models that more closely approximate the situation in humans.

Acknowledgments

The authors thank Drs. D. S. Goodman and H. N. Ginsberg for their critiques of this manuscript.

This work was funded by grants HL-21006 SCOR and HL-45095 from the National Heart, Blood, and Lung Institute and a grant from the Council for Tobacco Research, USA. I. J. Goldberg is the recipient of an Established Fellowship for Research from the American Heart Association, New York City Affiliate.

References

1. Eckel, R. H. 1989. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* 320:1060–1068.

2. Goldberg, I. J., N. A. Le, H. N. Ginsberg, R. M. Krauss, and F. T. Lindgren. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J. Clin. Invest.* 81:561–568.

3. Goldberg, I. J., W. S. Blaner, T. Vanni, M. Moukides, and R. Ramakrishnan. 1990. Role of lipoprotein lipase in the regulation of high density lipoprotein apolipoprotein metabolism. *J. Clin. Invest.* 86:463–473.

4. Kekki, M. 1980. Lipoprotein lipase action determining plasma high density lipoprotein cholesterol level in adult normolipaemics. *Atherosclerosis.* 37:143–150.

5. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* 60:473–485.

6. O'Brien, K., D. Gordon, S. Deeb, M. Ferguson, and A. Chait. 1991. Localization of lipoprotein lipase mRNA to foam cell-rich regions of human atherosclerotic plaques. *Clin. Res.* 39:335*a*. (Abstr.)

7. Yla-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. 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.

8. Jonasson, L., G. Bondjers, and G. K. Hansson. 1987. Lipoprotein lipase in atherosclerosis: its presence in smooth muscle cell and absence from macrophages. *J. Lipid Res.* 28:437–445.

9. Steinberg, D. 1983. Lipoproteins and atherosclerosis: a look back and a look ahead. *Arteriosclerosis.* 3:283–301.

10. Steinberg, D., S. Parthasarthy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320:915–924.

11. Bratzler, R. L., G. M. Chilsom, C. K. Colton, K. A. Smith, and R. S. Lees. 1977. The distribution of labeled low-density lipoproteins across the rabbit thoracic aorta in vivo. *Atherosclerosis.* 28:289–307.

12. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* 28:225–237.

13. Hoff, H. F., C. L. Heideman, R. L. Jackson, R. J. Bayardo, H.-S. Kim, and A. M. Gotto, Jr. 1975. Localization patterns of plasma apolipoproteins in human atherosclerotic lesions. *Circ. Res.* 37:72–79.

14. 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.

15. 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.

16. Saxena, U., M. G. Klein, and I. J. Goldberg. 1991. Lipoprotein lipase transport across endothelial cell monolayers. *Proc. Natl. Acad. Sci. USA.* 88:2254–2258.

17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345–1353.

18. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. 1. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* 260:212–221.

19. Shasby, D. M., and Shasby, S. S. 1985. Active transendothelial transport of albumin. Interstitium to lumen. *Circ. Res.* 57:903–908.

20. Shasby, D. M., L. L. Stoll, and A. A. Spector. 1987. Polarized metabolism of arachidonic acid by bovine aortic endothelial cell monolayers. *Am. J. Physiol.* 253:H1177–H1183.

21. Anderson, D. N., A. V. Nichols, T. M. Forte, and F. T. Lindgren. Particle distribution. *Biochim. Biophys. Acta.* 493:55–68.

22. Goldberg, I. J., J. J. Kandel, C. B. Blum, and H. N. Ginsberg. 1986. Association of plasma lipoproteins with post-heparin plasma lipase activities. *J. Clin. Invest.* 78:1523–1528.

23. Goldberg, I. J., D. A. Handley, T. Vanni, J. R. Paterniti, Jr., and J. A. Cornicelli. 1988. Membrane bound lipoprotein lipase on human monocyte-de-

rived macrophages: localization by immunocolloidal gold technique. *Biochim. Biophys. Acta.* 959:200–228.

24. Kirchgessner, T. G., K. L. Svenson, A. J. Lusis, and M. C. Schotz. 1987. The sequence of a cDNA encoding lipoprotein lipase. *J. Biol. Chem.* 262:8463–8466.

25. Cheng, C. F., G. M. Oosta, A. Bensadoun, A., and R. D. Rosenberg. 1981. Binding of lipoprotein lipase to endothelial cells in vitro. *J. Biol. Chem.* 24:12893–12898.

26. Chajek-Shaul, T., G. Friedman, G. Bengtsson-Olivecrona, I. Vlodavsky, and R. Bar-Sharvit. 1990. Interaction of lipoprotein lipase with subendothelial extracellular matrix. *Biochim. Biophys. Acta.* 1042:168–175.

27. Jackson, R. L., L. Socorro, G. M. Fletcher, and A. D. Cardin. 1985. Heparin binding to lipoprotein lipase and low density lipoproteins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 190:297–300.

28. Iverius, P.-H. 1972. The interaction between human plasma lipoproteins and connective tissues glycosaminoglycans. *J. Biol. Chem.* 247:2606–2613.

29. Weisgraber, K. H., and S. C. Rall, Jr. 1987. Human apolipoprotein B-100 heparin-binding sites. *J. Biol. Chem.* 262:11097–11103.

30. Radhakrishnamurthy, B., S. R. Srinivasan, P. Vijayagopal, and G. S. Berenson. 1990. Arterial wall proteoglycan-biological properties related to pathogenesis of atherosclerosis. *Eur. Heart J.* 11(Suppl. e):148–157.

31. Falcone, D. J., N. Mated, H. Shio, C. R. Minick, and S. D. Fowler. 1984. Lipoprotein-heparin-fibronectin–denatured collagen complexes enhance cholesteryl ester accumulation in macrophages. *J. Cell Biol.* 99:1266–1274.

32. Hurt-Camejo, E., G. Camejo, B. Rosengren, O. Wiklund, and G. Bondjers. 1990. Arterial proteoglycans increase the rate of oxidation of low density lipoproteins and its uptake by proteoglycan. *Arteriosclerosis.* 10:783a. (Abstr.)

33. Touny, S. E., W. Khan, and Y. Hannun. 1990. Regulation of platelet protein kinase C by oleic acid. *J. Biol. Chem.* 265:16437–16443.

34. Lynch, J. J., T. J. Ferro, F. A. Blumenstock, A. M. Brockenauer, and A. B. Malik. 1990. Increased endothelial albumin permeability mediated by protein kinase C activation. *J. Clin. Invest.* 85:1991–1998.

35. Hennig, B., D. M. Shasby, A. B. Fulton, and A. A. Spector. 1984. Exposure to free fatty acid increases the transfer of albumin across cultured endothelial monolayers. *Arteriosclerosis.* 4:489–497.

36. Chung, B. H., J. P. Segrest, K. Smith, F. M. Griffin, and C. G. Brouillette. 1989. Lipolytic surface remnants of triglyceride-rich lipoproteins are cytotoxic to macrophages but not in the presence of high density lipoproteins. A possible mechanism of atherogenesis? *J. Clin. Invest.* 83:1363–1374.

37. Gerrity, R. G. 1981. The role of the monocyte in atherosclerosis. I. transition of blood-borne monocytes into foam cells in fatty lesions. *Am. J. Pathol.* 103:181–190.

38. Ross, R. 1986. The pathogenesis of atherosclerosis—an update. *N. Engl. J. Med.* 314:488–500.

39. Goldstein, J. L., Y. K. Ho, M. S. Brown, T. L. Innerarity, and R. W. Mahley. 1980. Cholesteryl-ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine β-very low density lipoproteins. *J. Biol. Chem.* 255:1839–1848.

40. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* 52:223–261.

41. Traber, M. G., and H. J. Kayden. 1980. Low density lipoprotein receptor activity in human monocyte-derived macrophages and its relationship to atheromatous lesions. *Proc. Natl. Acad. Sci. USA.* 77:5466–5470.

42. Fogelman, A. M., I. Schechter, J. Seager, M. Hokom, J. S. Child, and P. A. Edwards. 1980. Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophages. *Proc. Natl. Acad. Sci. USA.* 77:2214–2218.

43. Basu, S. K., M. S. Brown, Y. K. Ho, and J. L. Goldstein. 1979. Degradation of low density lipoprotein–dextran sulfate complexes associated with deposition of cholesteryl esters in mouse macrophages. *J. Biol. Chem.* 254:7141–7146.

44. Suits, A. G., A. Chait, M. Aviram, and J. W. Heinecke. 1989. Phagocytosis of aggregated lipoprotein by macrophages: low density lipoprotein receptor–dependent foam cell formation. *Proc. Natl. Acad. Sci. USA.* 86:2713–2717.

45. Khoo, J. C., E. Mahoney, and J. L. Witztum. 1981. Secretion of lipoprotein lipase by macrophages in culture. *J. Biol. Chem.* 256:7105–7108.

46. Chait, A., P.-H. Iverius, and J. D. Brunzell. 1982. Lipoprotein lipase secretion by human monocyte–derived macrophages. *J. Clin. Invest.* 69:490–493.

47. Lindqvist, P., A. M. Ostlund-Lindqvist, J. L. Witztum, D. Steinberg, and J. A. Little. 1983. The role of lipoprotein lipase in the metabolism of trig'yceride-rich lipoproteins by macrophages. *J. Biol. Chem.* 258:9086–9092.