Lipoprotein lipase stimulates the binding and uptake of moderately oxidized low-density lipoprotein by J774 macrophages

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Lipoprotein lipase (LPL) stimulates the uptake of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) in different cell types, including macrophages, through bridging of LPL between lipoproteins and extracellular heparan sulphate proteoglycans (HSPG). Because macrophages produce LPL and because modified lipoproteins are present in the arterial wall *in vivo*, we wondered whether LPL also enhances the uptake of oxidized LDL by J774 macrophages. LDL samples with different degrees of oxidation, as evaluated by relative electrophoretic mobility (REM) as compared with native LDL are used, as well as native and acetylated LDL. Addition of 5 μg/ml LPL to the J774 cell culture medium stimulated the binding of both native LDL and moderately oxidized LDL (REM < 3.5) 50–100-fold, and their uptake was stimulated approx. 20-fold. The LPL-mediated binding of native LDL and moderately oxidized LDL was dose-dependent. Preincubation of the cells with heparinase (2.4 units/ml) inhibited the stimulatory effect of LPL, indicating that this LPL-mediated stimulation was due to bridging between the lipoproteins and HSPG. The binding to J774 macrophages of severely oxidized LDL (REM = 4.3) was stimulated less than 3-fold by LPL, whereas its uptake was not stimulated significantly. The binding and uptake of acetylated LDL (AcLDL) were not stimulated by LPL, although the LPL-molecule itself does bind to AcLDL. Measurements of the cellular lipid content showed that addition of LPL also stimulated the accumulation in the cells of cholesteryl ester derived from both native LDL and moderately oxidized LDL in a dose-dependent manner. We conclude that our results present experimental evidence for the hypothesis that LPL serves as an atherogenic component in the vessel wall.

INTRODUCTION

It has been shown that the enzyme lipoprotein lipase (LPL) enhances the cellular binding and uptake of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) by different cell types, including HepG2 cells, fibroblasts and THP-1 monocytes and macrophages. The increased binding and uptake of LDL and VLDL is due to the bridging of LPL between the lipoproteins and the heparan sulphate proteoglycans (HSPG) that are present on the plasma membrane [1–4].

If the proposed mechanism for LPL-mediated uptake of lipoproteins takes place in the liver, the role of LPL in lipoprotein uptake would be anti-atherogenic owing to the enhancement of the hepatic uptake of atherogenic lipoproteins such as LDL, VLDL and VLDL remnants. In contrast, in the intima of the vessel wall, LPL may serve as an atherogenic protein by stimulating the uptake of atherogenic lipoproteins by smooth muscle cells and macrophages, leading to foam cell formation. Thus LPL might have a dual function, depending on its location [5].

There are several reports showing that in the arterial wall LPL is associated with the atherosclerotic process. It has been reported that macrophages in atherosclerotic plaques synthesize LPL and that the concentration of LPL in the vessel wall is related to the concentration of cholesterol in the vessel wall [6]. Furthermore, smooth muscle cells, endothelial cells and macrophages present in the intima synthesize HSPG, depending on the amount of intracellular cholesteryl ester that has accumulated [7]. Cellular HSPG can bind apoB-containing lipoproteins, after which these lipoprotein–proteoglycan complexes can be taken up by macrophages mainly via a receptor-mediated pathway [8,9] and result in the formation of foam cells. Edwards et al. [10] suggest that in the atherosclerotic artery the LPL produced by macrophages and smooth muscle cells binds to the proteoglycans, thereby increasing the interaction of LDL with proteoglycans. Complex formation of LDL with HSPG leads to an increased oxidation of LDL, because of (i) a longer residence time of LDL complexes in the intima [11] and (ii) a higher oxidation rate of LDL–HSPG complexes than of free LDL [12]. Subsequently, increased oxidation of LDL results in an increased lipid accumulation in the macrophages, via the uptake of oxidized LDL by the scavenger receptor. Furthermore Ylä-Herttuala et al. [13] have shown that oxidized LDL is indeed present in atherosclerotic lesions *in vivo*.

All these facts together point to an important role for LPL in the atherosclerotic process. However, no studies have yet been made to determine whether LPL might also stimulate the binding and uptake of oxidatively modified LDL by macrophages directly. It also remains to be determined whether a stimulation of the binding of native LDL and possibly oxidized LDL, to macrophages is accompanied by an enhanced accumulation of cholesteryl esters in these cells. In the present study we found that in J774 macrophages LPL does indeed enhance the cellular binding and uptake of LDL that is partly oxidized. However, the uptake of severely oxidized LDL and acetylated LDL is not

Abbreviations used: AcLDL, acetylated low-density lipoprotein; CE, cholesteryl ester; DMEM, Dulbecco's modified Eagle's medium; FCS, foetal calf serum; HSA, human serum albumin; HSPG, heparan sulphate proteoglycan; LDL, low-density lipoprotein; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; OxLDL, oxidized low density lipoprotein; OxLDL2.2, moderately oxidized LDL; OxLDL4.3, severely oxidized LDL; REM, relative electrophoretic mobility; VLDL, very-low-density lipoprotein.

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stimulated by the presence of LPL. We also found that LPL stimulated the cellular cholesteryl ester content in a dose-dependent manner after incubation of the J774 macrophages with both native LDL and moderately oxidized LDL. Hence our findings provide experimental evidence for the hypothesis that in the vessel wall LPL serves as an atherogenic factor.

EXPERIMENTAL

Cells

Murine macrophage-like J774 cells were cultured in 75 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % (v/v) fetal calf serum (FCS), 0.85 g/l $NaHCO_3$, 4.76 g/l Hepes, 100 i.u./ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine. The cells were incubated at 37 °C in an atmosphere containing 5 % CO_2 in air. For each experiment, cells were plated in 12-well plates. The cells were fed every 3 d, and used for experiments within 7 d of plating. The cells were washed, 24 h before each experiment, with DMEM/1 % HSA and further incubated with DMEM containing 5 % (v/v) of lipoprotein-deficient serum (LPDS) instead of FCS.

Lipoproteins

Blood was obtained from healthy volunteers. Serum was separated from the cells by centrifugation at 1200 ***g*** for 10 min. LDL was isolated from the serum by density gradient ultracentrifugation by the method of Redgrave et al. [14]. The protein content of the LDL fraction was determined by the method of Lowry et al. [15]. After isolation, a part of the LDL sample was oxidized or acetylated as described below. LDL samples were iodinated by using the ^{125}I-monochloride method described by Bilheimer et al. [16]. The specific activity ranged from 50 to 300 c.p.m. per ng of protein. After iodination the LDL samples were dialysed extensively against PBS, pH 7.4, stored at 4 °C and used within 2 weeks.

Whenever unlabelled lipoproteins were used, immediately after isolation, dialysis against PBS and, subsequently, DMEM was performed at 4 °C.

LPDS was prepared by ultracentrifugation of the serum at $d = 1.21$ and removal of the lipoprotein-containing supernatant, followed by extensive dialysis against PBS.

Modification of LDL

Oxidation

LDL was oxidized by using $CuSO_4$. LDL (600 μl, 0.25 mg/ml), $CuSO_4$ (22.5 μl, 1.6 mM) and PBS (277.5 μl) were incubated at 37 °C. Various degrees of oxidation were obtained by inhibiting the oxidation reaction at different time points, using an excess of EDTA after 4, 8, 16 or 24 h of oxidation. Immediately after oxidation, lipoproteins were dialysed against PBS at 4 °C. The degree of oxidation was measured by means of agarose gel electrophoresis (100 V, 30 min, Paragon Lipoprotein Electrophoresis kit, Beckman Instruments), as shown in Figure 1. Subsequently the electrophoretic mobility relative to native LDL (REM) of the different oxidized LDL fractions (and VLDL and HDL as references) was determined.

Acetylation

LDL with known concentration (A ml, B mg of protein) was acetylated by using an equal volume (A ml) of saturated sodium acetate and 1.5B μl of acetic anhydride. The latter was added in portions of 2 μl for a period of 60 min, with continuous stirring on ice, and then dialysed extensively against PBS. The conversion of LDL to acetylated LDL (AcLDL) was confirmed by agarose gel electrophoresis (Figure 1), and the REM was determined subsequently.

Lipoprotein lipase

LPL was partly purified from fresh bovine milk by using heparin-Sepharose chromatography. After centrifugation (Sorvall GSA rotor, 30 min, 10000 ***g***, 4 °C), the skimmed milk was filtered and adjusted to 0.4 M NaCl. Heparin-Sepharose (CL-6B, Pharmacia), swollen according to the manufacturer's protocol, was equilibrated with 0.4 M NaCl and added to the milk. The mixture was shaken gently for 3 h at 4 °C. After being washed with 0.5 M NaCl and subsequently with 0.75 M NaCl, 10 mM KH_2PO_4, pH 6.8, the mixture was applied to a column and washed with 0.75 M NaCl, 10 mM KH_2PO_4, pH 6.8, at a flow rate of 0.3 ml/min. Thereafter, 1.5 M NaCl, 10 mM KH_2PO_4, pH 6.8, was used as an eluent buffer at a flow rate of 1.5 ml/min. LPL-containing fractions were pooled and an equal volume of 10 mM KH_2PO_4, pH 6.8, was added. The pooled fractions were applied to a 5 ml HiTrap Heparin affinity column (Pharmacia) equilibrated with 0.75 M NaCl, and eluted with a linear gradient of 0.75–2 M NaCl, 10 mM KH_2PO_4, pH 6.8, at a flow rate of 1 ml/min. The LPL-containing fractions were pooled and dialysed against 3.6 M $(NH_4)_2SO_4$, pH 6.8. The precipitated protein was collected after centrifugation (Sorvall SS-34 rotor, 30 min, 48000 ***g***, 4 °C), resuspended in 1–2 ml 20 mM NaH_2PO_4, 50 % glycerol and stored in aliquots at −80 °C.

Before the experiments, LPL was heat-inactivated by incubation in a water bath at 56 °C for 30 min.

Binding and uptake of lipoproteins by J774 macrophages

J774 macrophages were cultured in 12-well plates as described above. Then, 24 h before the start of the experiment, DMEM supplemented with 5 % (v/v) LPDS instead of FCS was added to the cells. The binding of ^{125}I-labelled LDL, oxidized LDL and acetylated LDL to the cells in the absence or in the presence of the indicated amounts of LPL, was determined after a 3 h incubation at 4 °C with 10 μg/ml of ^{125}I-labelled lipoprotein, either in the presence or in the absence of a 20-fold excess of unlabelled lipoprotein. The receptor-mediated (specific) cell-binding was calculated by subtracting the amount of labelled lipoproteins that was cell-bound after incubation in the presence of a 20-fold excess of unlabelled lipoprotein (non-specific) from the amount of labelled lipoprotein that was cell-bound after incubation in the absence of unlabelled lipoprotein (total cell binding). After removal of the medium the cells were washed four times with ice-cold PBS containing 0.1 % (w/v) BSA, and subsequently with PBS without BSA. Cells were then dissolved in 1 ml of 0.2 M NaOH. Protein content was measured by the method of Lowry et al. [15]. In an aliquot, the radioactivity represented the amount of cell-bound lipoprotein.

To measure the effect of LPL on the association and degradation of ^{125}I-labelled LDL, oxidized LDL and acetylated LDL, cells were incubated for 3 h at 37 °C with 10 μg/ml ^{125}I-labelled lipoprotein either in the absence or in the presence of 5 μg/ml LPL. At the end of the incubation period, a fraction of the medium was removed to determine of the amount of lipoprotein degraded, as described previously [17,18]. After removal of the rest of the medium, the cells were washed four times with ice-cold PBS/0.1 % (w/v) BSA, and subsequently with PBS without BSA. The cell-associated (bound plus internalized) lipoprotein

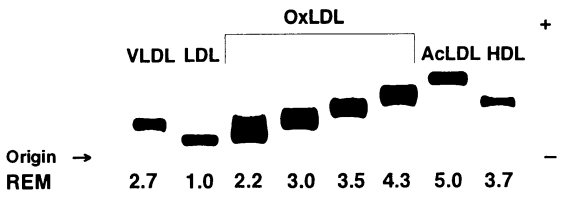

Figure 1 Agarose gel electrophoresis of different lipoproteins

LDL was oxidized or acetylated as described in the Experimental section. Native LDL, HDL and VLDL are shown as reference samples. The REM of each lipoprotein fraction was determined by measuring the distance from the origin to the centre of each band. REM increases with increasing oxidation time. Oxidized LDLs with REMs of 2.2, 3.0, 3.5 and 4.3 represent LDL that has been oxidized by $CuSO_4$ for 4, 8, 16 and 24 h respectively.

fraction was determined exactly as described previously [18]. In the respective figures, lipoprotein uptake is expressed as the sum of cell-associated and degraded lipoproteins.

Treatment with heparinase was performed by preincubating the cells for 2 h in the presence of 2.4 units/ml heparinase (Sigma, catalogue number H2519) at 37 °C before the experiment. The 2.4 units/ml heparinase was also present during the 3 h of incubation of the cells with labelled lipoproteins in order to prevent regeneration of HSPG on the cellular membrane during the experiment.

Binding of lipoproteins to plastic tissue-culture wells

After iodination, the lipoproteins were dissolved in DMEM/1 % HSA and incubated for 3 h in plastic tissue-culture wells without cells at 4 °C, either in the presence or in the absence of 5 μg/ml LPL. The wells had been incubated with DMEM/1 % HSA 24 h before the experiment. After 3 h, the binding of the lipoproteins to the plastic was measured as described above for binding to the cells.

Determination of the cellular lipid content

J774 cells were cultured in 6-well plates as described above. Then, 24 h before the start of the experiment, DMEM supplemented with 5 % (v/v) LPDS instead of FCS was added to the cells. At the start of the experiment, fresh DMEM media containing 1 % HSA (v/v) and 100 μg/ml lipoprotein either in the presence or in the absence of the indicated amounts of LPL were added to triplicate dishes of cells and incubated for 24 h at 37 °C. Control incubations were performed with DMEM/1 % HSA without any further additions. At the end of the incubation period, the cells were washed four times with 1.5 ml of PBS containing 0.1 % BSA, followed by one wash with PBS. Intracellular lipid content was determined as described by Havekes et al. [19]. Briefly, the cells were harvested by scraping with a rubber policeman and resuspended by three successive slow passages through a syringe needle (G25). Samples (100 μl) were taken for measurement of protein by the method of Lowry et al. [15]. Lipids were extracted from the cell suspension with methanol/chloroform (2:1, v/v) as described by Bligh and Dyer [20], after addition of cholesteryl acetate (2 μg) as an internal standard. The lipids were separated by using high-performance thin layer chromatography and the lipid bands were subsequently quantified densitometrically.

RESULTS

Effect of LPL on the binding and uptake of lipoproteins

LDL was oxidized to different degrees by incubation with $CuSO_4$ for different periods. For all experiments presented in this paper, LDL was isolated from the same subject to standardize the oxidation procedure. In Figure 1 it is shown that on agarose gel the electrophoretic mobility of LDL increases with oxidation time. AcLDL, VLDL and HDL are shown as reference samples. The REMs of the different lipoprotein samples, as compared with native LDL, are presented in Figure 1.

As can be seen in Table 1, 5 μg/ml LPL stimulated both the binding and uptake by J774 macrophages of native and oxidized LDL having an REM of 2.2 and 3.0 (OxLDL2.2 and OxLDL3 respectively). The binding of these lipoprotein fractions was stimulated 50–100-fold, and the uptake approximately 20-fold upon the addition of LPL. In contrast, the binding of oxidized LDL having REMs of 3.5 and 4.3 (OxLDL3.5 and OxLDL4.3) was stimulated by LPL by only 6-fold and 3-fold respectively, whereas their uptake was not stimulated significantly. In addition the binding and uptake of AcLDL were hardly stimulated by LPL, or not at all.

As has been shown before for fibroblasts [3], THP-1 macrophages [2] and HepG-2 cells [1,4], the LPL-mediated binding of LDL and OxLDL2.2 by J774 cells could be at least partly prevented by pretreating the cells with heparinase (Figure 2). This indicates that the stimulating effect of LPL in J774 cells is due to its bridging between HSPG and the lipoprotein particles.

We wondered whether LPL stimulates the binding of lipoproteins to cells in a dose-dependent way. Therefore the LPL-mediated binding of native LDL, moderately oxidized LDL and severely oxidized LDL to J774 cells was determined at LPL

Table 1 The effect of 5 μg/ml LPL on the binding and uptake of different ^{125}I-labelled lipoproteins by J774 cells

Lipoprotein binding and uptake (expressed as cell-associated plus degraded lipoprotein) were measured after a 3 h incubation of the cells with 10 μg/ml labelled lipoproteins at 4 °C and 37 °C respectively, either in the absence or in the presence of 5 μg/ml LPL. Specific binding and uptake are expressed as ng of labelled lipoprotein per mg of cell protein and were determined as described in the Experimental section. The values represent means ± S.D. for triplicate experiments.

	Binding (ng/mg cell protein)		Uptake (ng/mg cell protein)	
Lipoprotein	−LPL	+LPL	−LPL	+LPL
LDL	18.8 ± 5.9	1061.7 ± 80.6	39.2 ± 13.4	773.5 ± 91.7
OxLDL2.2	9.4 ± 7.1	991.1 ± 42.3	40.4 ± 15.9	744.5 ± 199.8
OxLDL3	14.5 ± 4.8	842.0 ± 79.0	36.2 ± 15.0	770.7 ± 75.2
OxLDL3.5	110.9 ± 12.5	651.0 ± 143.0	821.0 ± 285.0	988.2 ± 404
OxLDL4.3	154.2 ± 32.1	407.3 ± 71.8	1179.3 ± 236.1	1433.1 ± 202.1
AcLDL	217.3 ± 36.2	304.4 ± 12.5	5082.5 ± 382.4	5000.1 ± 646.4

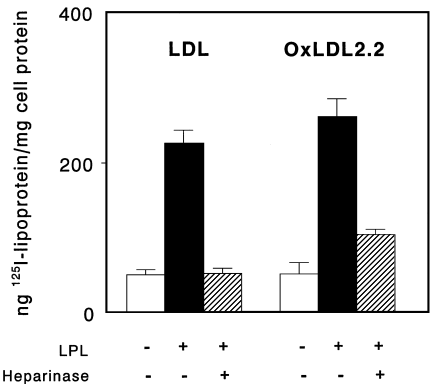

Figure 2 The effect of heparinase on LPL-mediated binding to J774 cells of ^{125}I-labelled LDL and ^{125}I-labelled OxLDL2.2

The cells were incubated for 3 h at 4 °C with 10 μg/ml ^{125}I-labelled LDL or ^{125}I-labelled OxLDL2.2 in the absence (open bars) or in the presence (filled bars) of 5 μg/ml LPL. For the heparinase treatment, the cells were preincubated for 2 h with 2.4 units/ml heparinase at 37 °C (hatched bars). The heparinase was also present during the 3 h incubation with labelled lipoproteins to prevent regeneration of HSPG. The presence or absence of LPL and heparinase is also indicated by + and − respectively. Specific binding was determined as described in the Experimental section. The values represent the means ± S.D. for triplicate experiments.

concentrations of 0, 1.25, 2.5 and 5 μg/ml. Figure 3 shows that increasing concentrations of LPL result in an increasing stimulation of the binding to J774 macrophages of both native LDL and moderately oxidized LDL (OxLDL2.2). For native LDL this dose-dependency is slightly stronger than for OxLDL2.2. The binding of severely oxidized LDL (OxLDL4.3) to J774 cells was not stimulated by LPL at any applied LPL concentration.

The results presented in Table 2 show that LPL stimulated the binding of native LDL to plastic, by bridging between the plastic and the LDL particle. Furthermore the binding to plastic of differently oxidized LDL and of AcLDL was also stimulated by LPL, although longer oxidation times resulted in a less pronounced stimulation.

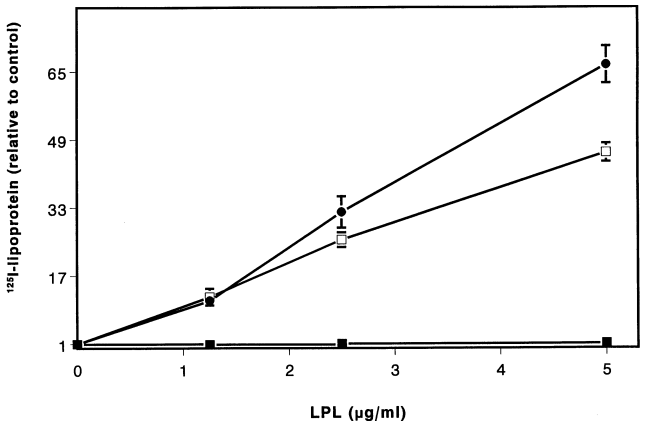

Figure 3 Dose–response relationship of LPL-mediated binding of different ^{125}I-labelled lipoproteins by J774 cells

Lipoprotein binding was measured after a 3 h incubation of the cells at 4 °C with 10 μg/ml ^{125}I-labelled LDL (●), ^{125}I-labelled OxLDL2.2 (□) or ^{125}I-labelled OxLDL4.3 (■) in the presence of 0, 1.25, 2.5 or 5 μg/ml LPL. The amount of lipoprotein bound in the presence of LPL is indicated relative to the amount of lipoprotein bound in the absence of LPL. The amount of lipoprotein bound in the absence of LPL was taken as 1 (control value). The amounts of binding in the absence of LPL are 13.2 ± 4, 14.5 ± 0.8 and 366 ± 27.1 ng/mg of cell protein for LDL, OxLDL2.2 and OxLDL4.3 respectively. Specific binding is expressed as ng of labelled lipoprotein per mg of cell protein and was determined as described in the Experimental section. The values represent the means ± S.D. for triplicate experiments.

Table 2 Effect of LPL on the binding of different ^{125}I-labelled lipoproteins to plastic

Cell-culture wells without cells were incubated with DMEM/1% HSA 24 h before the experiment. The wells were thereafter incubated for 3 h with 10 μg/ml ^{125}I-labelled lipoproteins dissolved in DMEM/1% HSA at 4 °C, in the presence or in the absence of 5 μg/ml LPL. Binding of the lipoproteins to the plastic of the well is expressed as ng of lipoprotein per well and measured as described in the Experimental section. Values are expressed as means ± S.D. for triplicate experiments.

	Lipoprotein (ng per well)					
	LDL	OxLDL2.2	OxLDL3	OxLDL3.5	OxLDL4.3	AcLDL
−LPL	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	1.0 ± 0.6	1.1 ± 0.1	1.3 ± 0.7
+LPL	640 ± 13	584 ± 17	420 ± 12	266 ± 7	36 ± 3	466 ± 10

Effect of LPL on the accumulation in J774 cells of cholesteryl esters derived from native and modified LDL samples

The effect of LPL on the cellular cholesterol content in J774 macrophages was determined after incubation of the cells with LDL, OxLDL2.2, OxLDL4.3 and AcLDL. Under all applied conditions, the cellular content of free cholesterol did not change significantly (results not shown). The results presented in Table 3 demonstrate that the cholesteryl ester (CE) accumulation in J774 macrophages after incubation of the cells with 100 μg/ml LDL is significantly increased in the presence of LPL, in a dose-dependent way. Similarly, LPL also significantly enhanced the accumulation of CE after incubation of the cells with OxLDL2.2. As expected, incubation of the J774 cells with 100 μg/ml OxLDL4.3 and AcLDL resulted in a marked increase in cellular cholesteryl ester content, which was not significantly influenced by simultaneous addition of LPL.

DISCUSSION

It is known that LPL stimulates the uptake of VLDL and LDL in different cell types, including HepG2 cells, fibroblasts and THP-1 monocytes and macrophages, owing to a bridging by LPL between LDL and HSPG [1–4]. It is also known that oxidized LDL is present in atherosclerotic lesions *in vivo*, and that it causes lipid accumulation in macrophages [21–24]. Because cells of the arterial wall, including macrophages, have been shown to produce and secrete LPL [6], we wondered whether LPL could also stimulate the uptake of oxidized LDL by macrophages, and whether that leads to increased cellular cholesteryl ester levels. Such an effect would imply that LPL serves as an atherogenic factor in the vessel wall.

In the present study, we showed that in J774 cells LPL stimulates the binding and uptake of both native LDL and moderately oxidized LDL 20–100-fold, owing to a bridging of LPL between HSPG and the lipoproteins. In contrast, the binding by J774 cells of severely oxidized LDL (REM $\geqslant$ 3.5) and acetylated LDL (REM = 5) was stimulated only minimally by LPL, whereas the subsequent uptake of these lipoproteins was not stimulated at all upon the addition of LPL. In accord with this, Obunike et al. [2] found that in THP-1 macrophages LPL caused a 3-fold increase in the binding of acetylated LDL,

Table 3 Effect of LPL on the cholesteryl ester content in J774 cells after incubation with different lipoproteins

Cellular cholesteryl ester content was measured after a 24 h incubation at 37 °C with 100 μg/ml lipoprotein in the absence or in the presence of the indicated amounts of LPL. Control incubations were performed in DMEM/1% HSA without any further additions. Cholesteryl ester content is expressed as μg of CE per mg of cell protein and was determined as described in the Experimental section. The values represent the means ± S.D. for triplicate experiments. * Significant difference between CE content after incubation in the presence of LPL and in the absence of LPL ($P < 0.05$, Student's *t*-test). N.D., not detected.

		Cholesteryl ester accumulated (μg per mg cell protein)				
LPL (μg/ml)	Lipoprotein...	Control	LDL	OxLDL2.2	OxLDL4.3	AcLDL
0		0.45 ± 0.03	0.93 ± 0.08	0.83 ± 0.06	8.83 ± 0.17	8.78 ± 0.63
5		N.D.	1.80 ± 0.39*	1.03 ± 0.28	8.38 ± 0.46	11.04 ± 2.79
10		N.D.	2.49 ± 0.33*	1.45 ± 0.24*	N.D.	N.D.

whereas LPL did not increase the degradation of acetylated LDL.

The absence of an LPL-mediated stimulation of the binding and uptake of severely oxidized LDL can be only partly explained by a defective binding of LPL to these lipoproteins, as indicated by the fact that increasing degrees of oxidation resulted in a decreasing ability of LPL to stimulate the binding of these lipoproteins to plastic (Table 2). This hypothesis is supported by the fact that apoB contains LPL-binding sites [25] that could be damaged by fractionation of apoB upon oxidation [26]. However, the fact that the binding of severely oxidized LDL (REM = 3.5) to plastic was still stimulated approx. 200-fold by LPL indicates that additional but unknown mechanisms underlie the absence of an LPL-mediated stimulation of the binding of severely oxidized LDL.

The lack of an LPL-mediated cellular binding and uptake of AcLDL cannot be explained by a defective binding of LPL to AcLDL, because LPL was able to stimulate its binding to plastic in a similar way to that of native LDL. We propose the following mechanism for the lack of an LPL-mediated binding and uptake of AcLDL and OxLDL3.5: LPL enhances the binding and uptake of LDL and moderately oxidized LDL (REM $\leqslant$ 3) by forming a bridge between the lipoprotein and the negatively charged HSPG, which are present on the plasma membrane. However, owing to the pronounced negative charges of both OxLDL3.5 and AcLDL (REM = 5), the complexes of LPL formed with these lipoproteins may not be able to bind to the negatively charged HSPG because of electrostatic repulsion forces.

To extrapolate the present results of LPL-mediated binding and uptake of lipoproteins to the situation *in vivo* in the vessel wall, it is necessary to speculate about the degree of oxidation of LDL in the atherosclerotic plaque. Steinbrecher and Lougheed [27] reported that LDL isolated from plaques or fatty streaks exhibited variable but usually only modest signs of oxidative change, including slightly increased electrophoretic mobility. Morton et al. [28] described an LDL-sized lipoprotein particle isolated from homogenates of human aortic atherosclerotic plaques, which migrated with a pre-beta electrophoretic mobility similar to that of VLDL (REM about 2). Similarly, Ylä-Herttuala et al. [13] also isolated lesion LDL with a relative electrophoretic mobility of 2 as compared with plasma LDL. These facts together suggest that it is highly unlikely that there is severely oxidized LDL *in vivo* with a relative electrophoretic mobility of more than 3. Thus it would be inappropriate to extrapolate the results obtained with severely oxidized LDL and AcLDL to the situation *in vivo* with regard to the effect of LPL.

The LPL-mediated binding of native LDL and moderately oxidized LDL was dose-dependent and occurred with LPL concentrations as low as about 1 μg/ml. According to Babirak et al. [29] the LPL mass in post-heparin plasma of normal controls was approx. 200 ng/ml. Several other groups have reported that macrophages synthesize LPL, and that the amount is related to the amount of intracellular cholesterol [6]. Ylä-Herttuala et al. [30] showed that in atherosclerotic lesions LPL protein is especially high in macrophage-rich intimal regions. Furthermore Goldberg et al. [31] showed that in addition to synthesis and secretion of LPL activity, monocyte-derived macrophages have LPL attached to their cell membranes. This suggests that the local concentration of LPL in atherosclerotic lesions may be much higher than the LPL concentrations found in plasma after heparin injection. Hence the results obtained in the present study may be relevant for the situation in the intima *in vivo*.

Although the uptake of native LDL and moderately oxidized LDL is low, even in the presence of LPL, compared with that of severely oxidized LDL and acetylated LDL (Table 1), our results indicate that the LPL-mediated binding and uptake by J774 cells of both native LDL and moderately oxidized LDL lead to a stimulation of cholesteryl ester accumulation in these cells (Table 3). From our results we therefore conclude that we have provided further experimental evidence for the hypothesis that LPL plays an important role in the formation of foam cells, a process that is considered to be one of the initial steps in atherogenesis.

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