Regulation of Lipoprotein Lipase Secretion in Murine Macrophages During Foam Cell Formation In Vitro

Effect of Triglyceride-Rich Lipoproteins

Orit Sofer, Menahem Fainaru, Zehava Schafer, and Rachel Goldman

Triglyceride-rich lipoproteins induce triglyceride accumulation in macrophages, leading to foam cell formation. The correlation between cell triglyceride accumulation and lipoprotein lipase (LPL) secretion in murine macrophages and the role that LPL plays in the accumulation process were examined. LPL secretion is defined as the extracellular LPL activity that accumulates during a 4-hour incubation of treated and untreated cells in a bovine serum albumin-containing RPMI-1640 medium. LPL secretion was suppressed (up to 70%) in a dose- and time-dependent manner when J774.1 cells were incubated with chylomicrons, very low density lipoproteins, and intermediate density lipoproteins but not with low or high density lipoproteins from normolipidemic and hypertriglyceridemic subjects. Oleic acid both suppressed LPL secretion and induced triglyceride accumulation. Suppression of LPL secretion preceded gross triglyceride accumulation, was reversible, and was not the result of a reduction in LPL mRNA. P388D1 cells neither secreted LPL nor accumulated triglyceride. Inhibition of LPL secretion by tunicamycin in both peritoneal macrophages and J774.1 cells prevented a hypertriglyceridemic very low density lipoprotein–induced triglyceride accumulation, an effect that was counteracted by addition of exogenous LPL. The results suggest that 1) extracellular hydrolysis of lipoprotein triglyceride is a major factor in inducing foam cell formation and 2) LPL secretion may be regulated by cell energy needs, and when these needs are exceeded, LPL secretion is suppressed.

KEY WORDS • chylomicrons • very low density lipoproteins • J774.1 cell line • tunicamycin • mRNA

Foam cells (lipid-laden cells) in the arterial wall, a characteristic component of atherosclerotic lesions, are derived to a considerable extent from macrophages.1,2 Both cholesteryl ester and triglycerides enter and accumulate in macrophages by several mechanisms, some of which involve cell surface receptors (for a review, see Reference 3). Macrophages in culture have been shown to express lipoprotein receptors for low density lipoproteins (LDLs), for chemically or biologically modified LDL (scavenger receptor), and for triglyceride-rich lipoproteins (TGRLs).3 Lipoprotein lipase (LPL) secreted by macrophages hydrolyzes triglycerides in chylomicrons and very low density lipoproteins (VLDLs), and the generated free fatty acids gain access to the cells, where they are metabolized or stored as triglycerides.4–6 The LPL-modified VLDLs and chylomicrons (VLDLs and chylomicron remnants) can be taken up by specific receptors on macrophages or other cell types, such as smooth muscle cells in atheromas, and lead to lipid accumulation.7 Thus, LPL secretion by macrophages can induce the accumulation of both triglyceride and cholesteryl ester in different types of cells.

Various aspects of the regulation of LPL synthesis and secretion in macrophages have been studied recently,8–14 and although a role for this enzyme in the transport and metabolism of TGRLs has been suggested,4–6,12 only a few studies have addressed the question about the effect of lipoproteins and cell triglyceride content on LPL secretion.15–18

In the present study we investigated the effect of human lipoproteins on the secretion of LPL and triglyceride accumulation in J774.1 cells, P388D1 cells, and thioglycolate-elicited peritoneal macrophages. The results establish a link between triglyceride accumulation and LPL secretion in murine macrophages. The triglyceride cell content regulates LPL secretion, whereas LPL synthesis and secretion are essential for triglyceride accumulation.

Methods

Media and Sera

RPMI-1640 and Dulbecco’s modified Eagle’s medium (DMEM) were from GIBCO, Grand Island, N.Y. Fetal calf serum (FCS) and horse serum were obtained from Bio-Lab, Jerusalem, Israel, and were heat inactivated (HI) for 30 minutes at 56°C.
Gentamicin (10 μg/ml), phenylmethylsulfonyl fluoride (1 mM) were added to the medium, and culture medium was replaced after 24 hours, and the cells were cultured for an additional 48 hours.

Other lipoproteins were isolated by ultracentrifuging the remaining plasma at different potassium bromide densities. This procedure was repeated twice. Other lipoproteins were isolated by ultracentrifuging the remaining plasma at different potassium bromide densities containing 1 mM Na₂EDTA in a 50.3 Ti rotor at pH 8.2. All centrifugations were carried out at 4°C for 18–20 hours (HDL was isolated over 44 hours). The lipoproteins were dialyzed in the dark for 24 hours against five changes of 100 volumes of 0.15 M NaCl and 1 mM Na₂EDTA (pH 8.2) at 4°C, stored in the dark at 4°C, and used within 7 days. The composition of the isolated lipoproteins is detailed in Table 1. Before use, the lipoproteins were diluted with RPMI-1640 containing 10% HI-FCS and sterilized by Millipore filtration (0.45 μm).

**Murine Macrophages**

J774.1 cells (a mouse lymphosarcoma-derived macrophage cell line) and P388D1 cells were serially passaged (biweekly) in RPMI-1640 supplemented with 10% HI-FCS (culture medium) and in DMEM supplemented with 10% HI horse serum, 1% x100 nonessential amino acids, and 1% glutamine (200 mM), respectively. All culture media were supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) (Bio-Lab), and all cell cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air. Thioglycolate-elicited peritoneal macrophages were collected from BALB/c male mice (8–12 weeks old) 4 days after intraperitoneal injection of 3 ml thioglycolate broth solution (DIFCO Laboratories, Detroit, Mich., 2.98 g/100 ml). The cells were seeded at 2×10⁵ macrophages per 35-mm plate, washed after 1 hour of adherence, and cultured in DMEM containing 10% HI-FCS and the antibiotics as specified above. Culture medium was replaced after 24 hours, and the cells were cultured for an additional 48 hours.

**Experimental Design**

For the experiments, adherent J774.1 cells and P388D1 cells were detached by mild streams of culture medium, washed, plated at 1.25×10⁶ cells (J774.1 cells) and 2.5×10⁵ cells (P388D1 cells) per 35-mm-diameter Falcon tissue-culture plates in the respective culture medium, and cultured for 72 hours and 96 hours.
respectively. At the specified time before culture termination, the medium was replaced by fresh culture medium containing the specified lipoproteins. Oleic acid was added, complexed to BSA in RPMI-1640 (without serum). At the end of the incubation the cells were washed three times with RPMI-1640 containing 0.5% BSA, a procedure that reduced membrane-bound LPL to undetectable levels. The cells were incubated for another 4 hours at 37°C in 0.8 ml RPMI-1640 containing 0.5% BSA (collection medium). Membrane-bound LPL was released at the end of the 4-hour incubation by a 3-minute treatment with heparin (0.04 units/ml) (Leo Pharmaceutical Products, Ballerup, Denmark). The enzyme collection media were transferred to tubes and kept on ice, and LPL activity was determined within 1 hour. LPL activity was stable in this medium at 4°C for several hours. Forty percent to 60% of the enzyme activity measured under the aforementioned experimental design was released spontaneously to the medium during the 4-hour incubation without heparin, and the rest was membrane bound and released by the 3-minute heparin treatment. When specified, heparin was included in the collection medium for the whole 4-hour collection period, thereby increasing LPL activity by 60–80% when compared with experiments in which heparin was added for the last 3 minutes, but the patterns of modulation of LPL secretion by lipoproteins were similar. After removal of the collection medium, cells were washed in Dulbecco’s phosphate-buffered saline (PBS), and cell protein was extracted in 0.1 M NaOH and determined by the method of Bradford.

Lipoprotein Lipase Assay

LPL activity was assayed by the method of Nilsson-Ehle and Schotz with minor modifications as described. Briefly, 200 μl collection medium was incubated with 100 μl of a substrate mixture, yielding a final concentration of 4.63 mM glycerol trioleate (containing glycerol tri[9,10(n)-3H]oleate, Amersham, Amersham, UK; specific activity was 90–130 dpm/nmol fatty acid), 0.45 mM lecithin, 1% BSA, 4.6% (vol/vol) glycerol, 50 mM tri(hydroxymethyl)aminomethane-hydrochloride (Tris-HCl pH 8.2), and 5.7% (vol/vol) HI horse serum. The substrate mixture was preincubated for 15 minutes at 37°C. The enzymatic reaction was carried out in a shaking water bath for 45 minutes at 37°C. One unit of enzyme activity was defined as that activity releasing 1 μmol of free fatty acid per milligram of cell protein. LPL activity was linear with enzyme concentration under the above assay conditions and was fully suppressed at 0.5 M NaCl. Intracellular LPL content assayed in cell homogenates as described was less than 5% of the secreted LPL under all conditions.

Extraction of Cell Lipids

Cell monolayers were washed three times in PBS containing 0.5% BSA and twice in PBS. Cell lipids were extracted with hexane/isopropanol alcohol (3:2, vol/vol) for 30 minutes at 23°C. The organic phase was removed, and the cells were further exposed to the same organic solvents, which were pooled and dried under N2.

Quantification of Cholesterol and Triglycerides

Cholesterol and triglyceride contents were determined in plasma, lipoproteins, and lipid cell extracts by using commercially available enzymatic kits (Boehringer-Mannheim, Mannheim, FRG). Lipoprotein protein was determined by the method of Lowry et al with BSA as the standard.

Oil Red O Staining

Cell monolayers were fixed with 2.5% glutaraldehyde in PBS for 20 minutes at 23°C and incubated with oil red O (a saturated solution in isopropanol diluted to 60% isopropanol) for 10 minutes at 23°C. The monolayers were then washed with water and counterstained with methylene blue.

RNA Extraction and Slot-Blot Analysis

RNA extraction was carried out essentially as described. J774.1 cells were cultured in 60-mm plates in RPMI-1640 containing 10% HI-FCS in the presence or absence of chylomicrons during the last 10 hours. The cells were scraped with a rubber policeman after addition of 1 ml PBS (4°C), centrifuged, and resuspended in 0.4 ml PBS. The recovered cells (4–5 x 10⁶ cells per plate) were pelleted and resuspended in 45 μl TE buffer (10 mM Tris and 1 mM EDTA, pH 7.4) containing 800 units/ml human placental RNAse inhibitor (Amersham). Ten microliters of 5% Nonidet P40 was added, and the pellets were resuspended and left on ice for 5 minutes. The nuclei were removed by centrifugation (12,000g, 2.5 minutes), and the supernatants were transferred into Eppendorf tubes containing 50 μl of a 40% formaldehyde–60% × 20 saline–sodium citrate buffer (SSC) solution. The tubes were incubated at 60°C for 60 minutes and kept at −80°C until analyzed. For slot-blot analysis, samples were diluted 1:11 in ×15 SSC, and then twofold serial dilutions were performed in ×15 SSC. Five serial dilutions of the aforementioned solutions were treated (the first slot of each sample contained RNA from about 1 x 10⁶ cells) onto a nylon membrane (Hybond N, Amersham) by using a slot-blot manifold apparatus. Each sample was blotted onto two separate membranes for probing LPL mRNA and β-actin mRNA. cDNA inserts radiolabeled by nick translation with deoxyctydine 5'-[32P]triphosphate were used as probes for LPL and β-actin. The membranes were prehybridized at 42°C for 24 hours in × 5 SSC, × 5 Denhardt’s solution, 0.05 M sodium phosphate (pH 6.5), 0.1% sodium dodecyl sulfate, 100 μg/ml denatured salmon sperm DNA, and 50% recrystallized formaldehyde. The [32P]cDNA probes (1 x 10⁶ cpm/ml) were hybridized to the blotted membranes in the above solution containing, in addition, 10% (wt/vol) dextran sulfate at 42°C for 36 hours. The blots were washed (30 minutes at 42°C) three times with × 3 SSC and twice with SSC and those with the β-actin probe were also washed twice with × 0.1 SSC, all containing 0.1% sodium dodecyl sulfate. The blots were exposed to Kodak XAR-5 x-ray film with an intensifying screen for 24 hours at −70°C. Autoradiographic images were quantified by scanning densitometry. A linear relation between the amount of loaded RNA and the LPL and actin signals was observed at the second, third, and fourth dilutions. The ratio of the signals of LPL and...
FIGURE 1. Time course plots of the effect of chylomicrons (panel A) and very low density lipoprotein (VLDL) (panel B) on lipoprotein lipase (LPL) secretion and triglyceride accumulation in J774.1 cells. Cells were cultured for 72 hours in RPMI-1640 containing 10% heat-inactivated fetal calf serum. Culture medium was replaced by fresh culture medium containing either chylomicrons (285 µg triglyceride per milliliter) or VLDL from a normolipidemic donor (172 µg triglyceride per milliliter) at the specified times before enzyme collection. The plates were washed three times with RPMI-1640 containing 0.5% bovine serum albumin and further incubated in the latter for 4 hours. Heparin (0.04 units/ml) was added for the last 3 minutes. The medium was removed, and LPL activity released into the medium was determined. The plates were washed twice in phosphate-buffered saline and treated with 0.1N NaOH, and cell protein was determined (—300 µg protein per plate). Control cultures treated at the various time points did not differ significantly from the zero point (not shown). Cell triglyceride content was determined in cell lipid extracts from parallel cultures after extensive washing (five times in phosphate-buffered saline) at the end of treatment with the lipoproteins. The results are expressed as mean±SD of three plates from a representative experiment.

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actin mRNA of the second and third dilutions was taken as an indicator of the relative amounts of LPL mRNA. The LPL cDNA probe (cDNA clone mL5, containing a 1.43-kb mouse LPL EcoRI insert) was a gift from Dr. M.C. Schotz,31 and the β-actin probe was a gift from Dr. L. Eisenbach.32

Results

Incubation of J774.1 cells with chylomicrons or normal VLDL resulted in a marked suppression of LPL secretion and a marked elevation of cell triglyceride. LPL secretion is arbitrarily equated with the extracellular enzyme activity that accumulates during a 4-hour incubation in BSA containing RPMI-1640, following the various specified treatments of the cells. The time course and dose response of the suppressive effect on LPL secretion and the enhancing effect on the accumulation of triglyceride are detailed in Figures 1 and 2, respectively. Figure 1 shows that incubation with chylomicrons or VLDL resulted in a 50% suppression of LPL secretion in about 2 hours and of 80% in 10 hours. Fifty percent inhibition of LPL secretion preceded the cell accumulation of triglyceride. The suppressive effect of chylomicrons and VLDL on LPL secretion was dose dependent (Figure 2); 50% suppression was reached at about 30 µg triglyceride per milliliter for the two lipoproteins (average from four experiments).

Quantification of cell triglyceride content showed that incubation with chylomicrons or normal VLDL led to a statistically significant increase in cell triglyceride content already at 4 hours. Triglyceride accumulation continued for at least 10 hours and did not reach saturation even at relatively high concentrations of chylomicrons or VLDL in the medium, conditions that led to a sevenfold increase in cell triglyceride content over the basal level. The effect of other lipoproteins from both normolipidemic and hypertriglyceridemic subjects on LPL secretion and triglyceride accumulation is detailed in Figure 3. Thus, in addition to chylomicrons, VLDL and intermediate density lipoprotein from both normolipidemic and hypertriglyceridemic individuals were also able to induce cell triglyceride accumulation. At the concentrations used (up to 500 µg protein per milliliter), neither LDL nor HDL from both normolipidemic and hypertriglyceridemic subjects had any appreciable effect on cell triglyceride content. The ability of plasma lipoproteins to suppress LPL secretion was closely related to their potential for inducing cell triglyceride accumulation.

Cell cholesterol content increased as much as threefold during incubation of J774.1 cells with lipoproteins,
Figure 3. Line plots showing effect of lipoproteins from hypertriglyceridemic (■) and normolipidemic (●) subjects on lipoprotein lipase (LPL) secretion (left panels) and triglyceride accumulation (right panels) in J774.1 cells. Culture medium was replaced with fresh culture medium containing the specified lipoproteins for the last 20 hours of culture. For experimental details, see the legend to Figure 1. Values are the mean of triplicate cultures ± SD from one representative experiment of two. VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

whereas cell triglyceride content increased as much as 50-fold. Cells incubated for 20 hours with chylomicrons (623 μg triglyceride per milliliter), VLDL (686 μg triglyceride per milliliter), hypertriglyceridemic VLDL (765 μg triglyceride per milliliter), and LDL (160 μg triglyceride per milliliter) increased their cholesterol content from 49 μg cholesterol per milligram of cell protein in control cultures to 79, 132, 108, and 59 μg cholesterol per milligram of cell protein, respectively, whereas the cell triglyceride content increased from 10 μg/mg cell protein in control cultures to 480, 300, 223, and 50 μg/mg cell protein, respectively. Morphological assessment (oil red O staining) of lipid accumulation in J774.1 cells incubated with TGRLs from both normolipidemic and hypertriglyceridemic individuals revealed that almost all of the cells were involved in foam cell formation.

Next we studied the reversibility of the effects induced by chylomicrons in J774.1 cells. The suppression of LPL secretion by a 10-hour incubation with chylomicrons was abolished within 4–6 hours of incubation of the cells with medium devoid of lipoproteins (before the 4-hour enzyme collection) (Figure 4A). The small overshoot of LPL secretion in chylomicron-treated cells when assayed 8 hours after chylomicron removal (+4 hours of enzyme collection) may reflect a beneficial effect of residual cell triglycerides on LPL secretion under conditions of medium deprivation of a fatty acid source. The rate of mobilization of cell triglyceride under the same conditions is given in Figure 4B. Cell triglyceride content decreased with time but was still twice the value of control cultures at 12 hours after the removal of chylomicrons.

Since the suppressive effect on LPL secretion did not seem to be limited to a specific lipoprotein and the data suggested a correlation with triglyceride accumulation, we explored whether oleic acid could affect LPL activity, cell triglyceride, or both. Oleic acid had a biphasic effect on LPL secretion (Figure 5). Control cells incubated in RPMI-1640 in the presence of 10% HI-FCS had an activity of about 42 units/mg cell protein (Figure 5). Incubation for 10 hours in RPMI-1640 containing 0.5% BSA and 3.3% BSA suppressed LPL secretory activity (28.0±0.9 and 4.5±1.5 units/mg cell protein, respectively). Incubation with increasing concentrations of oleic acid (up to 140 μM) restored the activity to 74–100% of that obtained with 10% HI-FCS. At higher concentrations (>75 μg/ml, or 417 μM), oleic acid led to suppression of LPL secretion (Figure 5). Cell triglyceride per milligram of cell protein was not affected by the change in the incubation medium from 10% HI-FCS to RPMI-1640 containing BSA with or without oleic acid, up to a concentration of 417 μM oleic acid in 0.5% BSA or 1.1 mM oleic acid in 3.3% BSA, at which concentration cell triglyceride content was doubled (from 16.1±1.5 to 32.8±3.1 μg/mg cell protein, respectively). Thus, the oleic acid-invoked suppression of LPL...
secretion preceded cell triglyceride accumulation in cultures incubated in oleic acid–3.3% BSA complexes.

Next we studied the possibility that the suppression of LPL secretion involved a reduction in cell LPL mRNA. Cells were incubated in the presence or absence of chylomicrons. The amount of LPL mRNA in J774.1 cells (normalized for cell actin mRNA) was not affected by the presence of chylomicrons in the medium at a concentration that suppressed LPL secretion up to 52% (Figure 6).

Thioglycolate-elicited peritoneal macrophages responded to incubation with chylomicrons (Figure 7) in a fashion similar to J774.1 cells, i.e., they suppressed LPL secretion and increased cell triglyceride content. Unlike J774.1 cells and peritoneal macrophages, P388D1 cells have a high basal triglyceride concentration (60–112 µg triglyceride per milliliter), which was not affected by inclusion of different sera (FCS, horse serum, and human serum) during culture. Incubating the latter cells with TGRLs at concentrations up to 900 µg triglyceride per milliliter medium did not lead to an increase in cell triglyceride content. P388D1 cells did not secrete LPL under all conditions tested (Figure 7).

The results presented suggest that in our experimental design, triglyceride accumulation may be dependent on LPL activity. To test this hypothesis we took advantage of tunicamycin, a known inhibitor of LPL secre-
free fatty acids, and tunicamycin does not affect cell triglyceride synthesis.

Discussion

The present study concentrated on the effect of human lipoproteins on triglyceride accumulation and LPL secretion in J774.1 cells. A positive correlation was established between the capacity of TGRLs to suppress LPL secretion and to induce triglyceride accumulation. Suppression of LPL secretion preceded appreciable triglyceride accumulation. The latter was not saturable at up to 10 hours and in a concentration range exceeding 300 μg triglyceride per milliliter. Chylomicrons and VLDL were shown to be substrates for LPL secreted from J774.1 cells. Moreover, LPL activity, although not a prerequisite for the saturable uptake of chylomicrons and VLDL (measured with radiolabeled particles), accelerated it. The amounts of triglyceride that accumulated under prolonged incubation with VLDL could not be accounted for on the basis of uptake of intact particles and were shown to be the result of extensive extracellular triglyceride hydrolysis, uptake of the free fatty acids, and the intracellular synthesis and storage of triglycerides. Our results corroborate this observation, in that treatment of the cells with tunicamycin, an inhibitor of LPL secretion, led to a reduced accumulation of triglyceride in cells incubated with VLDL. The fact that addition of exogenous LPL obviated the effect of tunicamycin suggests that under the experimental conditions used, most of the accumulating triglycerides are derived from the internalization of free fatty acids generated during extracellular hydrolysis of lipoprotein triglyceride. Judging by LPL activity released to the medium, we can assume that a high percentage of the triglyceride in the added chylomicrons or VLDL is hydrolyzed within the first few hours of incubation with the cells. Incubation with various lipoproteins, a triglyceride emulsion (Intralipid; data not shown), and oleic acid in the presence or absence of serum led to triglyceride accumulation to a level that was correlated with the concentration of triglyceride or oleic acid in the medium and not with the nature of the lipoproteins or added sera. This lends further support to the notion that LPL activity is essential for triglyceride accumulation in these cells in vitro. In this context it is of interest that in P388D1 cells, which have a high basal content of triglyceride and which do not express or secrete LPL as demonstrated here and by others, no further accumulation of triglyceride could be induced on incubation with any lipoprotein. The kinetics of LPL suppression and triglyceride accumulation suggest that the signals for the suppression of LPL synthesis precede massive accumulation of triglyceride and may be caused by an increased level of free fatty acids in the medium and cells or by other metabolic products that eventually lead to triglyceride accumulation. This suggestion is further supported by the observation that cessation of the flux of fatty acids into the cells by changing the medium to RPMI-1640 containing BSA led to the recovery of LPL secretion within 8 hours (4 hours of incubation and 4 hours of enzyme collection), a time at
which the cell triglyceride level was still elevated. The availability of free fatty acids to the cells seems to play a critical role in LPL secretion. Cell incubation for relatively extended periods (≥10 hours) with BSA resulted in a suppression of LPL secretion. This condition was shown in our experiments and in others' to induce the degradation of cell triglyceride and most probably to cause an efflux of fatty acids from the cells. A certain concentration of free oleic acid in equilibrium with oleic acid–BSA complexes was required for LPL secretion by J774.1 cells. The higher the BSA concentration, the higher the amount of oleic acid required before the cells could exhibit their LPL secretory activity and cellular triglyceride accumulation. The biphasic effect of extracellular oleic acid on LPL secretion suggests a feedback mechanism for the control of LPL secretion by one of its major products. In vitro and in vivo studies have shown that LPL has a built-in mechanism for product control at two other levels, i.e., the level of enzyme activity and the level of its availability on the endothelial cell surface. Fatty acids bind to the enzyme, thereby reducing its affinity to lipid droplets and abolishing enzyme activation by apolipoprotein CII. Fatty acids can displace the enzyme from its binding to heparin-agarose and to endothelial cells both in vivo and in vitro.

An apparent discrepancy exists between the reports that claim an increase in LPL secretion in macrophages on interaction with lipoproteins and the suppression observed in our studies. Different cell types were used in those studies (human monocytes and rat alveolar macrophages), and the experimental conditions varied to a large extent. Moreover, in the two reports from the same group, the cells were incubated with lipoproteins for extended periods, and enzyme collection was carried out in the presence of the lipoproteins. The enzyme collection medium did not contain heparin at any stage. Thus, in control cultures membrane-bound enzyme was probably not accounted for in the assay, whereas in lipoprotein-containing media both the lipoproteins and released fatty acids could lead to dissociation of the membrane-bound enzyme and result in an apparent increase in LPL secretion.

The pattern of LPL regulation is by and large similar to that described in heart cells. Heart cells in culture actively synthesize LPL. About 25% of the cellular activity is present at the cell surface and can be released by a 2-minute incubation with heparin. Unlike macrophages, however, heart cell cultures do not spontaneously secrete LPL into the medium. When cultures of rat heart cells were supplemented with rat plasma VLDL (75–750 μg triglyceride), a pronounced decrease (40–60%) in LPL activity occurred after 3–5 hours of incubation. The fall in LPL activity was mainly due to a fall in intracellular activity and not to a reduced activity of surface-bound enzyme. Similar to our observation, inhibition could be reproduced by increasing the fatty acid concentration of the medium. Furthermore, an in vivo reduction of plasma triglyceride levels by drug treatment resulted in an increase of LPL activity in the heart. The heart is largely dependent on fatty acids for energy production. The aforementioned considerations suggest that the level of LPL synthesis is regulated by both the availability of its substrate (triglyceride) and product (free fatty acids). The suppression of LPL secretion by chylomicrons was not accompanied by a reduction in cytoplasmic LPL mRNA in the treated cells. This suggests that either LPL mRNA in J774.1 cells has a rather long half-life (the cells were treated for 10 hours) or that chylomicrons exert their effect at a posttranscriptional stage. Stray et al have shown that treatment of human monocytes with actinomycin D for 2 hours did not affect LPL synthesis and that even a 24-hour treatment with the inhibitor resulted in only a partial inhibition (36%) of LPL activity. The authors suggested that the mRNA had a rather long half-life. Domin et al showed that in the human monocyte leukemia cell line THP-1, LPL mRNA levels decreased by only 50% after a 6-hour treatment with actinomycin D. Examples for the regulation of LPL secretion at both the transcriptional and posttranscriptional level are found in the literature. Interferon gamma and interleukin-2 reduced LPL mRNA in cultured human monocytes (after a 3-day treatment) by 42% and 53%, respectively.3 Tumor necrosis factor decreased LPL activity and LPL mRNA in 3T3-L1 cells and in rat and human adipose tissue in vitro. Insulin was found to stimulate an increase in LPL mRNA and an increase in the synthetic rate of LPL. On the other hand, an increase in LPL activity on feeding of obese subjects and rats seemed to be regulated at a posttranscriptional stage.

Macrophages require fatty acids as an energy source; they upregulate LPL secretion during proliferation and downregulate it during quiescence. Thus, it is possible that suppression of LPL secretion by lipoproteins reflects a control mechanism of the flux of fatty acids from the environment and their subsequent storage as triglyceride. J774.1 cells are proliferating cells. The phenomena described could be related to the regulation of energy metabolism in these cells. Therefore, we assessed LPL secretion versus triglyceride accumulation of LPL activity. The authors suggested that the mRNA had a rather long half-life. Domin et al showed that in the human monocyte leukemia cell line THP-1, LPL mRNA levels decreased by only 50% after a 6-hour treatment with actinomycin D. Examples for the regulation of LPL secretion at both the transcriptional and posttranscriptional level are found in the literature. Interferon gamma and interleukin-2 reduced LPL mRNA in cultured human monocytes (after a 3-day treatment) by 42% and 53%, respectively.3 Tumor necrosis factor decreased LPL activity and LPL mRNA in 3T3-L1 cells and in rat and human adipose tissue in vitro. Insulin was found to stimulate an increase in LPL mRNA and an increase in the synthetic rate of LPL. On the other hand, an increase in LPL activity on feeding of obese subjects and rats seemed to be regulated at a posttranscriptional stage.

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References

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