Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells

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During fasting, a reduction in lipoprotein lipase (LPL) activity has been observed in rat fat pad with no change in enzyme mass, whereas LPL mRNA and synthesis are increased, suggesting that insulin and/or fatty acids (FA) regulate LPL activity post-translationaly [Doolittle, Ben-Zeev, Elovson, Martin and Kirchgessner (1990) J. Biol. Chem. **265**, 4570–4577]. To examine the role of FA, either preadipose Ob1771 cells or Ob1771 and 3T3-F442A adipose cells were exposed to long-chain FA and to 2-bromopalmitate, a non-metabolized FA. A rapid (2–8 h) and dose-dependent increase (up to 6-fold) in LPL mRNA occurred, primarily due to increased transcription, which is accompanied by a decrease (down to 4-fold) in LPL cellular activity. Under these conditions, secretion of active LPL was nearly abolished. Removal of FA led to full recovery of LPL activity. LPL gene expression in 3T3-C2 fibroblasts was not affected by FA treatment. However fatty acid-activated receptor transfected-3T3-C2 cells, which show FA responsiveness, had increased LPL gene expression upon FA addition. LPL synthesis and cellular content appeared unaffected by FA treatment, whereas secretion of LPL was inhibited. These results indicate that FA regulate the post-translational processing of LPL. It is proposed that the regulation of LPL activity by FA is important with regard to the fine-tuning of FA entry into adipocytes during fasting/feeding periods.

INTRODUCTION

Lipoprotein lipase (LPL) is synthesized and secreted by adipocytes. After transport to the endothelial cell surface, the enzyme hydrolyses triacylglycerol-rich lipoprotein particles. LPL bound to heparan proteoglycans turns over very rapidly and a continual replenishment of the enzyme occurs. Consequently, numerous possibilities exist with regard to the control of LPL activity on its way to its site of action, including pre- and post-translational events, intracellular transport, secretion from adipocytes, transport and binding to the luminal surface of the endothelium. The activity of LPL in adipose tissue is known to be regulated by nutritional and hormonal factors [1,2]. Under catabolic conditions, Doolittle et al. [3] observed that fasting produced a reduction in LPL catalytic activity in rat fat pads with no change in enzyme mass such that LPL specific activity was decreased. This was accompanied by an increase in LPL mRNA content and LPL synthesis rate. Other reports showed that catecholamines, which may also stimulate lipolysis and increased production of released fatty acids (FA), decreased LPL synthesis, degradation and secretion in rat adipocytes whereas LPL mRNA levels were either affected or were not [4–6]. High-fat feeding of diabetics, accompanied by increased FA levels, led to impairment of insulin-mediated restoration of LPL activity [7]. In contrast, insulin supplementation decreased FA levels and restored the LPL activity of adipose tissue [8,9]. Altogether, these observations point out the inverse correlation between FA levels and LPL activity. Recent reports have shown that FA can activate within a few hours the expression of FA-related genes, i.e. a fatty acid transporter, adipocyte fatty acid-binding protein (A-FABP) and acyl-CoA synthetase [10–12]. FA also trigger the terminal differentiation of preadipose to adipose cells [13]. These effects of FA have been shown to be first mediated by a fatty acid-activated receptor (FAAR) [10]. The effective concentration of FA was likely to be low within the cells since it equilibrates with the FA levels unbound to serum albumin [14], which are within the concentration range 5–15 nM [15]. Moreover, the metabolically relevant pool of intracellular unbound FA should be only a very small percentage of cell FA since this pool should be in equilibrium with FA bound to epidermal fatty acid-binding protein (E-FABP) in preadipose cells [16] and to E-FABP and A-FABP in adipose cells [17]. Thus, low levels of exogenous unbound FA can chronically up-regulate A-FABP gene expression, and this phenomenon is reversible upon FA removal [11,12]. A search for evidence of FA control of LPL has shown that the main product of triglyceride hydrolysis exerts a feedback control on its activity [18], can cause dissociation of the enzyme from its binding to endothelial heparan sulphate [19] and can also cause a decrease of LPL activity in cultured rat adipocyte precursors [20]. These observations suggest that various regulatory mechanisms may operate in preventing intracellular overloading of adipose and muscle tissues by FA. Since long-chain FA are able in adipose cells to modulate the expression of lipid-related genes, we decided to study the regulation of the expression of the LPL gene in preadipose and adipose cells by FA. The results presented herein indicate that FA increase LPL mRNA but decrease LPL activity and nearly abolish heparin-released LPL. The phenomenon is reversible and reflects both translational and post-translational control as protein synthesis and the steady-state level of LPL appear unaffected. This regulation may provide an additional mechanism for the fine-tuning of FA entry into preadipose and adipose cells.

Abbreviations used: A-FABP, adipocyte fatty acid-binding protein; DMEM, Dulbecco's modified Eagle's medium; E-FABP, epidermal fatty acid-binding protein; FA, fatty acids; FAAR, fatty acid-activated receptor; GPDH, glycerol-3-phosphate dehydrogenase; LPL, lipoprotein lipase; PPARγ_2, peroxisome proliferator, activator receptor γ_2.

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EXPERIMENTAL

Cell culture

Ob1771 [21], 3T3-F442A [22], 3T3-C_2 [23] and 3T3-C_2/FAAR27 [10] cells were plated at a density of 2×10^3 cells per cm^2 and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% bovine serum, 200 units/ml of penicillin, 50 μg/ml of streptomycin, 33 μM biotin, and 17 μM pantothenate (termed standard medium). Confluence (day 0) was reached within 5 days. Differentiation of Ob1771 and 3T3-F442A cells was obtained by shifting the confluent cells into DMEM containing 8% fetal calf serum, 17 nM insulin and 2 nM tri-iodothyronine (termed differentiation medium). Media were changed every other day. FA were dissolved in dimethyl sulphoxide at a concentration of 50 mM and aliquots of the stock solution were added to standard or differentiation medium in order to obtain the final FA concentration. This medium was pre-warmed at 37 °C for 45 min before addition to the cells. The actual concentration of total FA in bovine serum was found to be about 0.6 mM (results not shown), corresponding to about 48 μM in culture medium. Thus, in most cases the final FA concentration varied from 53 μM (5 μM added) to 148 μM (100 μM added). Assuming that the serum concentration of albumin is normally about 0.6 mM [24], the FA-to-albumin molar ratio varied from 1 to 3, values which are within physiological levels. Under these conditions, the actual concentrations of unbound FA varied from 10 to 80 nM [15].

RNA analysis

RNAs were prepared according to Chomczynski and Sacchi [25] and analysed as previously described [26].

Preparation of cell lysates

At the end of experiments, cells were washed twice at 4 °C with PBS, pH 7.4, solubilized for 30 min at 4 °C in 5 mM sodium barbital buffer, pH 7.4, 1 M glycerol and 150 mM NaCl (buffer A) containing 0.2 % (w/v) Triton X-114 and 3 μg/ml heparin. Cell lysates were treated at 30 °C to pellet Triton X-114 micelles [27]. Supernatants were used for LPL activity assays.

Preparation of secreted enzyme

Ob1771 adipocytes were treated or not with 50 μM 2-bromopalmitate for 24 h. Cells were then washed with the differentiation medium at 37 °C and further incubated in the same medium containing 3 μg/ml heparin for 15 or 30 min. In the case of treated cells, the concentration of 2-bromopalmitate was decreased to 5 μM in the secretion medium. The secretion media were then collected, rapidly chilled at 0 °C and diluted 1.5-fold with buffer A as previously described [28]. Control experiments showed that, under these conditions, inclusion of 2 μM 2-bromopalmitate in the LPL assay did not decrease its activity by more than 5%.

Enzymic assay of LPL

The activity is taken as the the apoC-II-dependent hydrolysis of tri-[9,10-^{3}H]oleoyl glycerol (950 TBq/mol). Assay conditions have been described previously [29].

Immunoblot analysis

Differentiated Ob1771 cells were maintained for 24 h in the absence or presence of either 200 μM α-linolenate or 50 μM 2-bromopalmitate. Cells from two pooled 60-mm dishes were then washed twice with PBS at 4 °C and homogenized in 500 μl of 20 mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.1 mM PMSF and 4 μg/ml pepstatin, using a Potter–Elvehjem homogenizer (20 strokes). After centrifugation of the homogenate at 6000 ***g*** for 10 min at 4 °C, the supernatant was treated with Triton X-114 as described for the preparation of cell lysates. Proteins (25 μg) were separated by SDS/PAGE and immunoblotting was performed as already described [28]. The blots were visualized by the Amersham enhanced chemiluminescence (ECL) system.

Immunoprecipitation assays

Before labelling, differentiated Ob1771 or 3T3-F442A cells were treated or not with 50 μM of 2-bromopalmitate for 24 h. The cells were then washed in methionine-free DMEM containing 8% dialysed fetal calf serum and labelled during the last 30 min in the same medium containing 2 μM methionine and 1.85 MBq of [^{35}S]methionine per ml. For cellular LPL, lysis of [^{35}S]methionine-labelled cells, immunoprecipitation of cell lysates and analysis of immunoprecipitates were performed as previously described [28]. For heparin-released LPL, the cells were washed at the end of the labelling period with the differentiation medium at 37 °C and further incubated in the presence of 3 μg/ml heparin with or without 50 μM 2-bromopalmitate for 15 min. Immunoprecipitation and analysis of immunoprecipitates were performed as described above for cell lysates

Nuclear transcription assays

Isolation of nuclei and nuclear transcription assays have been described previously [21]. Briefly, incubation of 10^7 nuclei for 30 min at 37 °C in the presence of 7.4 MBq of [α-^{32}P]UTP led to the incorporation of about 10^5 c.p.m./μg of input DNA. ^{32}P-Labelled reaction products (10^7 c.p.m.) were hybridized for 48 h at 65 °C to Hybond C extra membranes containing 10 μg of denatured cDNA probes. The washed membranes were exposed at -70 °C to Hyperfilm MP Amersham with intensifying screens. Results were quantified by densitometry using an LKB Ultroscan XL laser densitometer.

Materials

Culture media and fetal calf serum were obtained from Gibco (Cergy-Pontoise, France). 2-Bromopalmitate was from Aldrich (Strasbourg, France) and FA, bovine serum and other chemical products were purchased from Sigma (St. Quentin, France). Radioactive materials and Hybond membranes were from Amersham (Les Ulis, France) and the random priming kit was from Boehringer Mannheim (Meylan, France).

RESULTS

Regulation by FA of LPL gene expression in preadipose cells

In order to evaluate the effects of FA in preadipose cells, time-course experiments were performed in various cell lines. One-day post-confluent Ob1771 cells were already committed to differentiate at this time, since they were expressing the early markers pOb24/A2COL6 and LPL mRNAs [11,30,31]. However, cells were not yet terminally differentiated since they did not express late markers such as ALBP, acyl-CoA synthetase and glycerol-3-phosphate dehydrogenase (GPDH) mRNAs [10,11]. When exposed to 250 μM α-linolenate as a natural FA or 100 μM 2-bromopalmitate as a non-metabolized FA [12], the LPL mRNA content of Ob1771 preadipose cells increased more than 2-fold

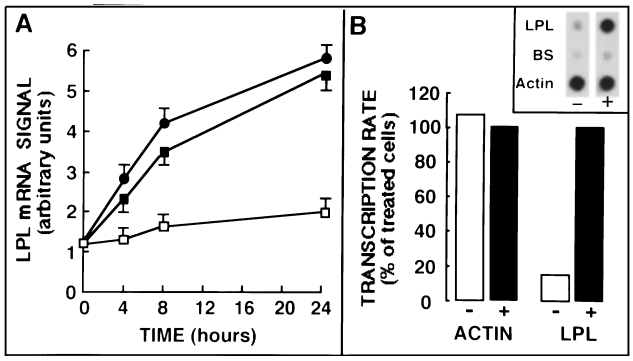

Figure 1 Effect of α-linolenate and 2-bromopalmitate on the expression of LPL mRNAs in Ob1771 preadipocytes

(**A**) One-day post-confluent Ob1771 preadipocytes were maintained in the standard medium in the absence (□) or the presence of either 250 μM of α-linolenate (■) or 100 μM of 2-bromopalmitate (●) for the indicated times. RNAs were prepared and analysed as described in the Experimental section. The results were normalized to β-actin signals. The values are reported as means ± ranges for four independent experiments. (**B**) Run-on assays from nuclei of one-day post-confluent Ob1771 cells exposed (+) or not (−) for 24 h to 100 μM of 2-bromopalmitate. The results are expressed by taking as 100% the signal value obtained for each probe with nuclei of FA-treated cells and subtracting the low background level determined with plasmid Bluescript (BS).

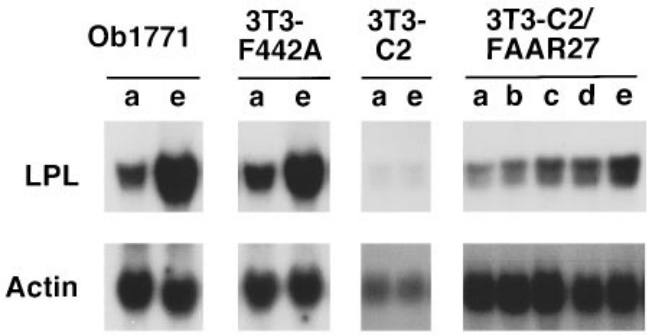

Figure 2 Effect of 2-bromopalmitate on LPL mRNA content in cells of different clonal lines

One-day post-confluent Ob1771, 3T3-F442A, 3T3-C_2 and 3T3-C_2/FAAR27 cells were maintained in standard medium, in the absence (lane a) or the presence of 3 μM (lane b), 10 μM (lane c), 30 μM (lane d) or 100 μM (lane e) 2-bromopalmitate. RNAs were prepared and analysed as described in the Experimental section. The autoradiogram shown is representative of three experiments performed with different series of cells. Exposure times for LPL were 15 h for Ob1771 and 3T3-F442A cells and 24 h for 3T3-C_2 and 3T3-C_2/FAAR27 cells.

within 4 h and approx. 6-fold within 24 h (Figure 1A), reaching a plateau within 48 h (results not shown). Nuclear run-on experiments were carried out using nuclei from Ob1771 preadipose cells maintained for 24 h in the absence or the presence of 100 μM of 2-bromopalmitate. The results in Figure 1B indicate that the LPL transcription rate, which was very low in untreated cells, was strongly increased (7-fold) by FA treatment, indicating that this modulation was primarily due to transcriptional activation of the LPL gene. As in Ob1771 preadipose cells, this phenomenon is observed in 3T3-F442A preadipose cells, whereas the weak signal detected for LPL mRNA in 3T3-C_2 fibroblasts used as control cells, remained unchanged (Figure 2). Since we have reported recently that stable transfection of 3T3-C_2 fibroblasts with an FAAR expression vector confer FA inducibility of

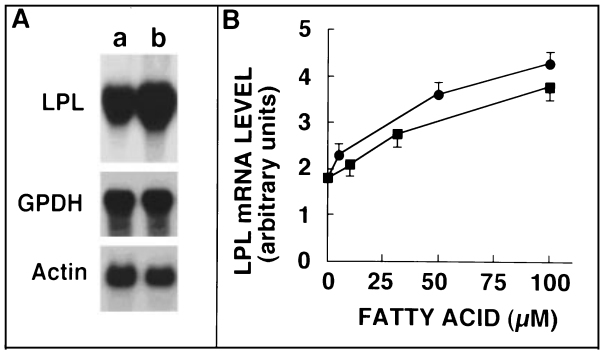

Figure 3 Effect of FA on the expression of LPL mRNA in Ob1771 adipocytes

(**A**) Total RNA (20 μg/lane) from twelve-day post-confluent Ob1771 adipocytes treated (lane b) or not (lane a) for 24 h with 100 μM 2-bromopalmitate were analysed as described in the legend to Figure 2. (**B**) Ob1771 adipocytes maintained in differentiation medium were exposed for 24 h to increasing concentrations of 2-bromopalmitate (●) or α-linolenate (■). RNAs were analysed as described in the legend to Figure 1A. The values are reported as means ± ranges for three independent experiments.

A-FABP and FA transporter genes [10], additional experiments were performed with regard to the expression of the LPL gene. Northern blots showed that the LPL gene responds in 3T3-C_2/FAAR-27 cells in a dose-dependent manner to 3–100 μM 2-bromopalmitate (Figure 2). Similar results were obtained with two other independent FAAR-expressing clones whereas this phenomenon did not take place in 3T3-C_2 cells stably transfected with an empty expression vector (results not shown).

Regulation by FA of LPL gene expression in adipose cells

In order to separate the effects of FA on the expression of LPL gene *per se* from their more general effect on the differentiation of preadipose to adipose cells, which is accompanied by an increase in LPL mRNA and LPL activity [26,30], similar experiments to those described above were performed in fully differentiated Ob1771 adipose cells. The results in Figure 3B also show a positive but weaker effect of α-linolenate and 2-bromopalmitate in adipose cells compared with preadipose cells with respect to LPL mRNA content. The effect of FA was confined to LPL as the content of GPDH mRNA remained unchanged (Figure 3A) as did the GPDH activity levels (1200 ± 110 m-units/mg). In both preadipose and adipose cells, 2-bromopalmitate was effective within a similar range of concentrations (Figure 3B).

Regulation by FA of LPL activity in preadipose and adipose cells

LPL gene expression is clearly enhanced by FA in preadipose and adipose cells (Figures 1–3). Thus, it was of the utmost importance to evaluate whether LPL activity was affected similarly, as contradictory results have been reported. For instance, upon exposure of chicken adipocytes to long-chain polyunsaturated FA, LPL mRNA and LPL activity decreased in parallel [32], whereas, upon exposure of 3T3-L1 adipose cells to high concentrations of retinoic acid, which is also an FA, LPL activity decreased but LPL mRNA remained unchanged [33]. Time-course experiments (Figure 4A) showed, that upon exposure to 250 μM α-linolenate or 100 μM 2-bromopalmitate, there was a ~4-fold decrease in LPL activity in Ob1771 preadipose cells within 24 h, under conditions where a 6-fold

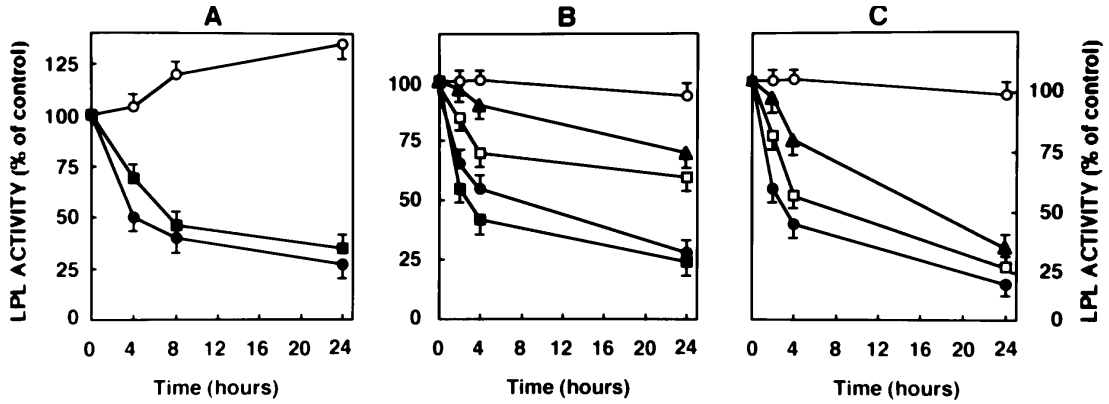

Figure 4 Time-course of LPL activity in response to α-linolenate and 2-bromopalmitate

(**A**) One-day post-confluent Ob1771 preadipocytes were maintained in standard medium in the absence (○) or the presence of either 250 μM α-linolenate (■) or 100 μM 2-bromopalmitate (●) for the indicated times. LPL activity was then measured as described in the Experimental section. The activity values at time zero were 2.7 ± 0.3 m-units/mg. (**B** and **C**) Twelve-day post-confluent Ob1771 adipocytes were maintained for the indicated times in differentiation medium in the absence (○) or the presence of different concentrations of α-linolenate (**B**): 10 μM (▲), 30 μM (□), 100 μM (●) and 250 μM (■); or 2-bromopalmitate (**C**): 5 μM (▲), 50 μM (□) and 100 μM (●). LPL activities were determined from duplicate 60-mm dishes. The activity values at time zero varied from 15 to 24 m-units/mg, depending upon the percentage of differentiated cells observed in three different series of cells.

Table 1 Effect of 2-bromopalmitate on LPL secretion

Fourteen-day post-confluent Ob1771 adipocytes were maintained in 60-mm dishes for 24 h in the differentiation medium in the absence (control) or the presence of 50 μM 2-bromopalmitate. Cellular LPL activities and heparin-released LPL activities during either a 15 min or a 30 min incubation period were determined from duplicate dishes as described in the Experimental section. The values are reported as means ± range for three independent experiments.

	LPL activity (m-units/dish)		
		Heparin-released LPL	
Condition	Cellular	15 min	30 min
Control	41.90 ± 3.80	34.50 ± 3.10	58.20 ± 5.45
2-Bromopalmitate	10.60 ± 1.80	0.60 ± 0.10	2.55 ± 0.30

Table 2 Relationships between the nature of FA and LPL activity in Ob177 cells

Ob1771 adipocytes, after 12 days exposure to differentiation medium, were treated for 4 h with 100 μM of the various FA as indicated. LPL activity was determined from duplicate dishes and mean values are reported. The number of experiments is given in parentheses.

Condition	LPL activity (% of control)
Control	100 (6)
Caprylic acid	88 ± 5 (2)
Capric acid	91 ± 5 (2)
Lauric acid	82 ± 6 (2)
Myristic acid	56 ± 6 (2)
Palmitic acid	58 ± 5 (2)
Stearic acid	56 ± 6 (2)
Oleic acid	55 ± 5 (2)
α-Linolenic acid	54 ± 5 (6)
Arachidonic acid	56 ± 6 (2)
2-Bromocaprylic acid	80 ± 6 (2)
2-Bromopalmitic acid	42 ± 4 (6)

increase in LPL mRNA content was observed (Figure 1A). A similar phenomenon occurred in Ob1771 adipose cells: α-linolenate (Figure 4B) and 2-bromopalmitate (Figure 4C) provoked a dose-dependent decrease of LPL activity, with a 4-fold decrease following treatment for 24 h with 100 μM 2-bromopalmitate. This effect was fully reversible, since, after treating Ob1771 adipose cells with 100 μM of 2-bromopalmitate, under conditions where LPL activity was decreased 4-fold, complete recovery of LPL activity was obtained within 4 h following FA removal. It should be pointed out that total cell protein levels remained similar in the absence or presence of FA (1.65 ± 0.15 mg/60-mm dish).

In addition to the effects of FA on LPL cellular activity, their most striking effect was to prevent almost all LPL secretion from Ob1771 adipose cells despite the fact that a significant cellular LPL activity (~ 25%) remained present in these cells (Table 1). The lack of heparin-released LPL upon heparin exposure was not due to the inhibitory action on LPL activity of the high concentrations of FA present in the culture medium, since this medium was replaced for secretion by a medium containing heparin (3 μg/ml) and 5 μM 2-bromopalmitate and since addition of 2 μM 2-bromopalmitate to lysates and medium containing released enzyme from untreated cells did not alter LPL activity by more than 5%.

Relationships between LPL activity and FA

As shown in Table 2, the potency to decrease LPL activity was clearly dependent upon the length of the fatty acyl chain. Short-chain and middle-chain FA, including the non-metabolized 2-bromocaprylate, were ineffective, whereas long-chain FA exerted potent effects, including the non-metabolized 2-bromopalmitate. The influence of the degree of unsaturation of the fatty acyl chain appeared to be less important, as stearate, oleate and α-linolenate showed similar potency. Arachidonic acid and α-linolenic acid were no more potent than oleic acid, suggesting that metabolites, which could originate from γ- or α-linolenic acid metabolism, were not likely to be the mediators responsible for the inhibitory effect on LPL activity.

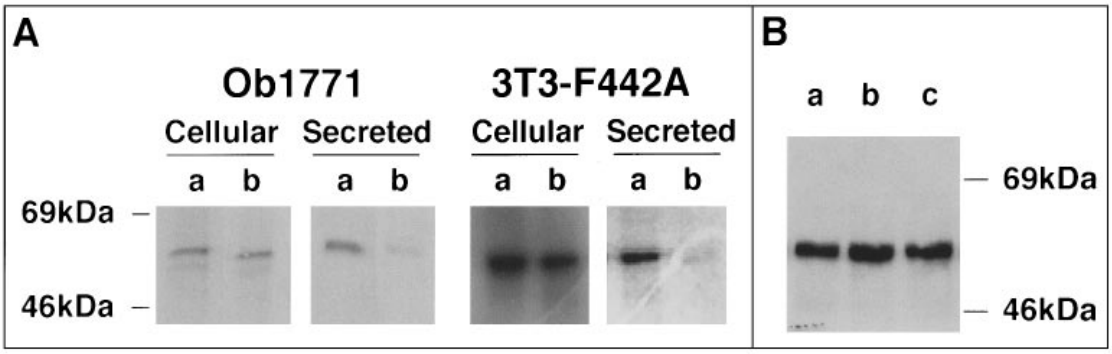

Figure 5 LPL protein levels in adipocytes treated or not with FA

(**A**) Differentiated Ob1771 or 3T3-F442A cells were untreated (lane a) or treated for 24 h with 50 μM of 2-bromopalmitate (lane b). The cells were labelled with [^{35}S]methionine for 30 min. Secretion media and cell lysates were prepared and subjected to immunoprecipitation as described in the Experimental section. Each lane corresponds for each condition to one quarter of cell lysate or secretion medium from cells maintained in 60-mm dishes. (**B**) Fourteen-day post-confluent Ob1771 adipocytes were maintained in the differentiation medium in the absence (lane a) or the presence of either 50 μM 2-bromopalmitate (lane b) or 200 μM α-linolenate (lane c) for 24 h. Cell lysates (25 μg of protein) were analysed for LPL by immunoblotting as described in the Experimental section. The results are representative of three independent experiments performed with different series of cells.

LPL protein levels in adipose cells treated or not with FA

To evaluate further the mechanisms underlying the effect of FA on LPL synthesis, fully differentiated adipose cells were pulse-labelled with [^{35}S]methionine according to the method of Vannier et al. [28]. Total protein synthesis was not altered under these conditions (1.2×10^7 c.p.m./mg of protein), thus excluding the possibility that FA inhibition of LPL activity was due to inhibition of protein synthesis. The [^{35}S]methionine-labelled LPL was immunoprecipitated from whole-cell lysates and also from media after incubation of the cells for 15 min in the presence of heparin. The results in Figure 5A show no difference between control and treated cells from both Ob1771 and 3T3-F442A clonal lines. As expected from the results shown in Table 2, where there is no activity in the secretion medium of FA-treated cells, labelled LPL remained undetectable but was detectable in the secretion medium of untreated cells. Analysis of the steady-state levels of LPL by immunoblotting (Figure 5B) led to the observation that the LPL protein content of Ob1771 adipose cells was unaffected by 24 h treatment with FA, indicating no significant difference in the synthesis and degradation of LPL between untreated and FA-treated cells.

DISCUSSION

These studies document that LPL gene expression and LPL activity in adipose cells are influenced by administration of long-chain FA. LPL gene expression is increased whereas LPL activity is decreased under conditions where LPL protein content remains similar. The effect of FA appears to be more dramatic on LPL secreted activity than on LPL cellular activity. Administration from 2 to 24 h of $C_{18:2,\ n-3}$ (α-linolenate), $C_{20:0,\ n-6}$ (arachidonate) and $C_{18:1,\ n-9}$ (oleate) FA was sufficient to provoke effects on the cellular and heparin-released activities (Figure 4), in contrast with the heparin-released LPL protein from chicken adipocytes which requires from 1 to 8 days of exposure and responds more strongly to $n-3$ and $n-6$ FA than to $n-9$ FA [32]. Previous studies have shown that the most important regulation of adipose tissue LPL takes place post-translationally. In all instances, the changes in LPL activity were more pronounced than the changes in LPL mRNA content [2,3,5,32,34]. In adipocytes from fasted rats, the major LPL species was sensitive to endo H, suggesting that the high-mannose form of LPL was catalytically less active than the mature complex oligosaccharide form of the enzyme [3]. Since LPL is known to play the role of a 'gatekeeper' in controlling FA entry in adipose tissue, we decided to examine the fate of LPL mRNA, LPL protein and LPL activity upon exposure of adipose cells to FA. Clearly, LPL mRNA levels can be modulated positively by these nutrients both in preadipose (Figures 1 and 2) and adipose cells (Figure 3) from different clonal lines. This regulation occurs primarily at the transcriptional level (Figure 1B). Among the *trans*-acting factors which control LPL gene expression in response to FA, FAAR is likely to play a critical role via FAAR/RXR heterodimers [9] recognizing putative peroxisome proliferator responsive elements which are present within the human LPL promoter (J. Auwerx, personal communication). It should be pointed out that, under the conditions in Figure 2, no trace of peroxisome proliferator activator receptor γ_2 (PPARγ2) was present in Ob1771 and 3T3-C_2/FAAR27 cells [10], excluding a role in preadipose cells for PPARγ2 [35] in LPL gene expression. In differentiated adipose cells where both FAAR and PPARγ2 are expressed [10,35], a role for both factors appears likely. However, despite an increase in LPL mRNA content, a net decrease in LPL activity was observed (Figure 4). This regulation occurred only in the presence of long-chain FA (Table 2), suggesting the physiological relevance of this phenomenon if one recalls the low concentrations of short- and medium-chain FA in plasma lipoproteins and their main oxidative fate in liver and muscle tissue. The physiological relevance of the FA effect is also supported by the observation that the FA-mediated decrease in LPL secretion was more pronounced than the decrease in active LPL (Table 1). In order to evaluate whether translational and/or post-translational mechanisms could explain these results, LPL protein synthesis and LPL content were examined by [^{35}S]methionine labelling followed by immunoprecipitation and immunoblot analysis. No difference was observed between control and FA-treated cells using both approaches (Figure 5), strongly suggesting that LPL protein synthesis and degradation were not significantly changed upon exposure to FA. Since LPL mRNA content appears to be increased under these conditions (Figure 3), it is assumed that FA exert some inhibitory effect on the translation of LPL mRNA. Furthermore, LPL activity is decreased whereas total cell protein and LPL protein remains unchanged in FA-treated cells, implying a decrease in LPL specific activity upon exposure

of the cells to FA. The fact that no heparin-released LPL protein could be detected would indicate that LPL was prevented from undergoing intracellular trafficking and release in FA-treated cells. Since the trimming of terminal glucose residues from the high-mannose form present in the rough endoplasmic reticulum was essential for further processing and secretion, it can be postulated that FA regulate this critical step [36]. LPL activity has been shown to be regulated by FA, i.e. by product inhibition [18] and release from heparan proteoglycan binding sites [19]. It is tempting to postulate that the additional mechanism reported herein provides, by means of a post-translational mechanism, a fine-tuning of FA entry into adipocytes during fasting/feeding periods where FA fluxes within adipose tissue undergo profound changes. In this respect, since the albumin concentration is approx. 0.2 mM in the interstitial fluid surrounding adipocytes *in vivo*, the FA-to-albumin ratio is likely to be high at the time when hydrolysis of triglycerides from chylomicrons or very-low-density lipoproteins is taking place in adipose tissue [37]. Our study suggests that LPL is then subject to feedback control by its main product at a post-translational level, decreasing its activity and secretion from adipocytes.

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