Forms of lipoprotein lipase in rat tissues: in adipose tissue the proportion of inactive lipase increases on fasting

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Previous studies have shown that the ratio of lipoprotein lipase (LPL) catalytic activity to LPL mass in tissues differs in different conditions, but it is not clear whether this occurs by a change in the catalytic efficiency of the LPL molecules, or because of a shift in the relation between active and inactive forms of the enzyme. To explore this, we have measured LPL activity and mass in detergent extracts of rat tissues. LPL specific activity was high and similar in heart, skeletal muscle, lung and brain. The liver had significantly lower specific activity, which is in accord with previous findings that the liver takes up and catabolizes LPL. The specific activity was also low in adipose tissue from fasted rats. When tissue extracts were applied to columns of heparin–agarose and eluted by a gradient of NaCl, a peak of active LPL was eluted at 1.0 M NaCl, but there was also a peak of inactive LPL protein, which was eluted at 0.6 M NaCl. In adipose tissue, LPL activity decreased by 70–80 % during an overnight fast, whereas LPL mass decreased by only 20–40 %. The mass ratio between inactive and active LPL, as separated by heparin–agarose chromatography, increased from 0.5 to over 2 during the fast. In hearts there was no significant difference between fed and fasted rats in total LPL activity, LPL mass or in the distribution between inactive and active forms. The results indicate that the relation between inactive (probably monomeric) and active (dimeric) forms of LPL is a target for post-translational regulation in adipose tissue.

INTRODUCTION

Lipoprotein lipase (LPL) is synthesized in parenchymal cells of several extrahepatic tissues (e.g. heart, muscle and adipose tissue) and is transferred to the local vascular endothelium, where it hydrolyses triacylglycerols in chylomicrons and very-low-density lipoproteins [1]. Several studies have shown that the ratio of LPL catalytic activity to LPL mass in tissues differs in different conditions, e.g. during development [2], with the nutritional state [3,4] and on induction of diabetes [5,6]. It is not clear, however, if this occurs by a change in the catalytic efficiency of the LPL molecules, or because of a shift in the relation between active and inactive forms of the enzyme.

Functional LPL is a dimer of two identical subunits [7]. Monomerization is associated with a conformational change and irreversible loss of catalytic activity [8]. During culture of 3T3-L1 adipocytes, which continuously secrete LPL, it was observed that relatively large amounts of inactive LPL accumulated as insoluble aggregates [9]. In expression systems for recombinant LPL, some of the enzyme appears as inactive monomers [10]. In plasma, most of the LPL is inactive and associated with lipoproteins [11]. Thus there are inactive as well as active forms of LPL in biological systems.

To study what the situation is in rat tissues *in vivo*, we first ascertained that the methods that we proposed to use would extract and measure all forms of LPL from the tissues. Then we measured LPL mass and activity in a number of tissues. The results showed that the specific activity was high and similar in many of the tissues studied, suggesting that the active form predominated. Two notable exceptions were the liver, and the adipose tissue from fasted rats. That most of the lipase in liver would be inactive was expected from previous studies, which have shown that the liver takes up, inactivates and degrades LPL [12]. For adipose tissue, previous studies have indicated that there is post-transcriptional regulation of the enzyme [3,13–16]. To study this further, we fractionated adipose-tissue extracts by chromatography on heparin–agarose columns [17–21]. The results demonstrated a shift from predominantly active, dimeric, LPL in the fed state to predominantly inactive, monomeric, LPL in the fasted state. In contrast, no such shift occurred in the heart.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing around 150 g were purchased from Möllegaard Breeding Centre (Ejby, Denmark). The animals were acclimatized for at least 10 days before each experiment and had by then reached a weight of around 200 g. They were maintained on standard laboratory chow and had free access to water. Fasted rats were deprived of food at 17:00 h the day before the experiments. All animals used in each experiment were killed between 08:00 and 09:00 h by decapitation. Animal procedures were approved by the animal ethics committee at Umeå University.

Materials

Leupeptin and pepstatin were from the Peptide Institute (Osaka, Japan). BSA and aprotinin (Trasylol) were from Sigma (St. Louis, MO, U.S.A.). Heparin–agarose was prepared as previously described [22]. Heparin used in buffers was from Lövens (Malmö, Sweden). Microtitre plates for the ELISA were from Nunc, Roskilde, Denmark. Intralipid (10 %) was from Pharmacia Hospital Care, Stockholm, Sweden.

Buffers

Buffer A, used for homogenization of tissues, contained 0.025 M NH_3, BSA (1 mg/ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), Trasylol (25 IU/ml), 5 mM EDTA, heparin (5 IU/ml), 1 % Triton X-100 and 0.1 % SDS, pH 8.2. Buffer B, used to dilute

Abbreviation used: LPL, lipoprotein lipase.

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samples for the ELISA, contained 10 mM sodium phosphate with 150 mM NaCl (PBS), 0.05 % Tween-20, 1 mg of heparin/ml and 4 mg of BSA/ml. Buffer C, used to rinse plates in the ELISA, contained 10 mM PBS and 0.05 % Tween-20. Buffer D, used to elute and wash the heparin–agarose columns, contained 20 mM Tris/HCl, pH 7.4, 20 % glycerol, 0.1 % Triton X-100 and 1 mg of BSA/ml.

Tissue preparations

Tissue samples (0.3–1.0 g) were placed in ice-cold buffer A, and homogenized with a Polytron homogenizer (PT-MR 3000; Kinematica AG, Switzerland). Adipose-tissue samples were always from epididymal fat-pads. The homogenates were centrifuged at 3000 rev./min for 10 min, after which the supernatants were collected. To check the efficacy of the extraction, the pellet was dispersed in 1 % SDS in the same buffer but without Triton X-100, and heated at 80 °C for 10 min. This extract was then diluted in 10 vol. of the same buffer with 1.1 % Triton X-100 without SDS to obtain the same detergent concentrations as in the original buffer. After centrifugation, the supernatant was assayed in the ELISA. Less than 2 % of total immunoreactive LPL was found in the second extract, demonstrating that the first extraction was quite efficient. Control experiments showed that LPL activity decreased by approx. 20 % in tissue homogenates over an 8 h period at 4 °C, but the immunoreactivity was not affected. Therefore all activity measurements were done immediately on fresh homogenates. Fractions from the heparin–agarose columns were kept on ice for less than 1 h before they were assayed for activity. Samples to be assayed for immunoreactivity were either frozen or measured immediately. Immunoreactivity was not affected by repeated freezing and thawing cycles.

Antibodies

A chicken polyclonal antibody (chicken 132) was raised against bovine LPL as previously described [23]. Antibodies were isolated from egg yolks and were affinity-purified by using an LPL–agarose column [11]. The antibodies were eluted with 0.2 M glycine, pH 2.7, and 50 mM diethylamine, pH 12, and immediately dialysed against 10 mM Tris/HCl, pH 7.4, overnight. The 5D2 monoclonal antibody (Mab) against bovine LPL [24] was kindly given by Dr. John D. Brunzell, University of Washington, Seattle, WA, U.S.A. For detection in the ELISA, a commercial goat anti-mouse IgG antibody conjugated with horseradish peroxidase (A-2554, Sigma), was used. The anti-(hepatic lipase) antibody was raised in a rabbit [25], and immunoglobulins were purified on a column of Protein A–agarose (Pharmacia).

Activity measurements

Intralipid into which ^{3}H-labelled triolein had been incorporated by sonication was used as substrate [26]. Briefly, 2 μl of sample was incubated with substrate in the presence of 10 μl of heat-inactivated rat serum (as source of apolipoprotein CII) and 6 % (w/v) BSA in a total volume of 200 μl. The assay was linear with time and amount of enzyme within the range used (see the Results section). The assay temperature was 25 °C; 1 m-unit of enzyme activity corresponds to 1 nmol of fatty acid released/min. All samples were assayed in duplicate. As an internal standard, rat post-heparin plasma was used. The activity of hepatic lipase in plasma and liver was suppressed by preincubation for 2 h with rabbit anti-(hepatic lipase) IgG. Values from the assay were corrected against the internal standard. An average value for the standard was calculated from results of eight separate incubations. The standard was stored frozen at −70 °C. One sample was thawed for each assay and then discarded.

Immunoreactivity measurements

For this study, a sandwich ELISA, using affinity-purified IgG from chicken 132 for capture of the antigen and the 5D2 Mab for detection, was developed. Antibodies from several immunized chickens were tested. About one in ten of the chickens produced antibodies with high immunoreactivity against LPL. The wells were coated with 100 μl of the 132 antibody (5 mg/ml in PBS) and incubated for 4 h at 37 °C. The plates were washed twice with buffer C. Then 100 μl of the samples diluted in buffer B was applied to the coated wells. A standard for the ELISA was prepared by chromatography of rat post-heparin plasma on heparin–agarose. The column was eluted with a salt gradient in buffer D. Fractions containing the peak of LPL activity, around 1 M NaCl, were combined and frozen in small aliquots. The LPL mass was calculated from an assumed specific activity of 0.40 m-unit/ng, a value typical for bovine and human LPL [11]. This standard could be thawed repeatedly without loss of immunoreactivity. The same standard was used during the whole study. The standard was diluted in the same way as the samples, and 100 μl was added to the wells. The plates were incubated overnight at 4 °C. After washing four times with buffer C, 100 μl of the 5D2 Mab (diluted 1:10000 in 10 mM PBS, 0.1 % Tween-20, 4 % BSA) was added to the wells, and the plates were then incubated for 3 h at room temperature. After another four washes with buffer C, 100 μl of the A-2554 anti-mouse IgG antibody conjugated with horseradish peroxidase (diluted 1:8000 in 10 mM PBS, 0.05 % Tween-20, 4 % BSA) was added to the wells and incubated at room temperature for 2 h. After four washes with buffer C, 100 μl of the substrate (0.4 mg of 1,2-*o*-phenylenediamine and 0.4 μl of H_2O_2/ml in a citrate buffer at pH 5.0) was added to the wells. After development for 10–15 min, the A_{490} was measured in a Thermomax microplate reader. Three dilutions of each sample were made, and the values that did not fall within the range of the standard curve were discarded.

Heparin–agarose affinity chromatography

To separate active and inactive forms of LPL, tissues were homogenized in buffer A, but without heparin, and centrifuged as described above. They were then applied to a column of heparin–agarose. The column was washed for 20 min at a flow rate of 1 ml/min with buffer D at 4 °C, and was then eluted by a 70 + 70 ml gradient of 0–1.6 M NaCl in buffer D. Fractions (5 ml) were collected, and samples were immediately assayed for catalytic activity and immunoreactivity as described above. To be able to process a larger number of tissue samples, a batch elution technique was developed where the NaCl gradient was replaced by a single pass of 8 ml of buffer D containing 0.6 M NaCl, followed by a single pass of 8 ml of buffer D containing 1.6 M NaCl. This resulted in two fractions containing inactive and active forms of LPL, respectively. Control experiments showed that the separation was almost complete by this procedure.

RESULTS

Assay validations

Incubation of increasing amounts of tissue homogenates revealed that the LPL activity assay was not linear (Figure 1), except with low amounts of sample. This was not due to inhibition by the detergent-containing buffer, since no inhibition was seen with

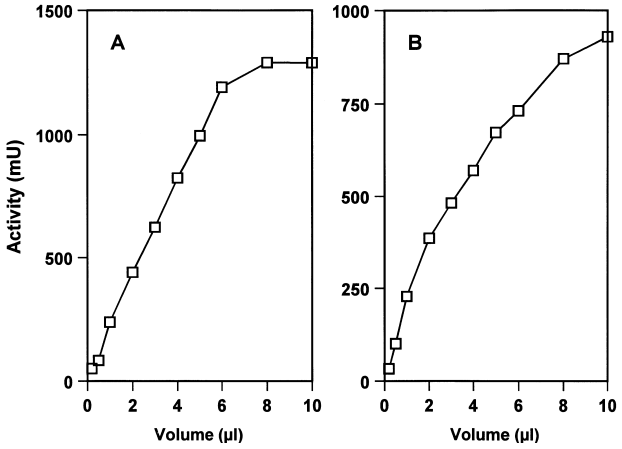

Figure 1 Linearity of the activity assay

Increasing amounts of homogenates from adipose tissue (**A**) and heart (**B**) were incubated for 60 min as described in the Materials and methods section. The total volume in each incubation tube was 200 μl. Data points are means of triplicate incubations. Abbreviation: mU, m-units.

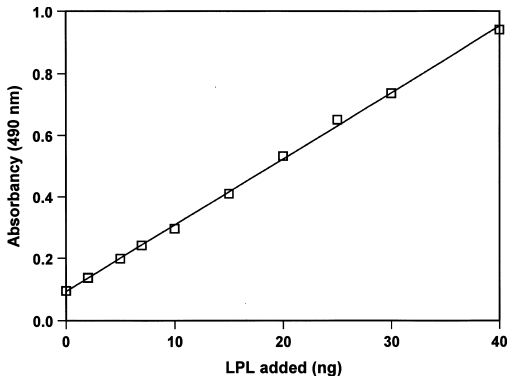

Figure 2 Standard curve for purified rat LPL in the ELISA

LPL standard was generated by affinity chromatography of rat post-heparin plasma as described in the Materials and methods section. Data points are means of duplicate samples.

volumes of buffer up to 50 μl added to the assay. The non-linearity was most pronounced with heart extracts and was probably due to non-specific inhibition by the extracted proteins. Therefore, in the following, the extracts were suitably diluted, and volumes corresponding to 2 μl or less were used. The assay was linear with time to at least 180 min (results not shown). In the following we used an incubation time of 60 min in all experiments. The mean within-assay covariance was 3 %, and the inter-assay covariance, as determined from 8 assays of the post-heparin-plasma standard, was 14 %. In the ELISA we used IgG from chicken 132 to capture the LPL antigen. Similar antibodies were previously shown to react with active and inactive forms of both bovine and human LPL [23]. Initial studies showed that antibodies from chicken 132 cross-reacted well also with rat LPL. The 5D2 monoclonal antibody used for detection has been shown by Peterson et al. [17] and Vilella et al. [11] to react efficiently with inactive monomeric, active dimeric and denatured forms of LPL. This ELISA will measure the number of LPL subunits. Thus the mass of LPL does not depend on whether LPL is in a monomeric or a dimeric form. Figure 2 shows a dose–response curve for the sample of LPL semi-purified from

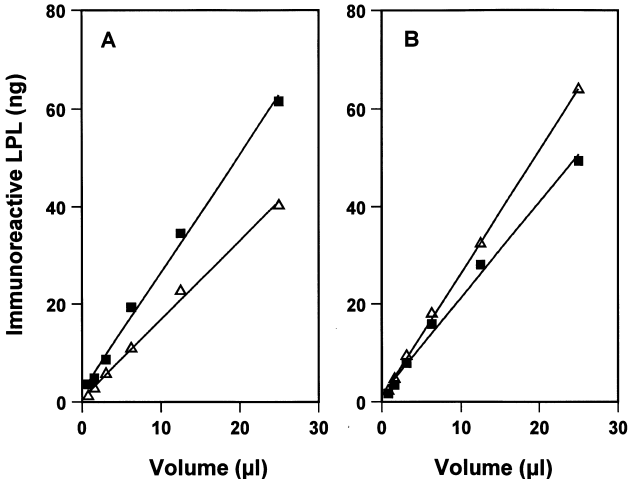

Figure 3 Linearity of the reactivity of LPL in tissue homogenates in the ELISA

Samples of epididymal adipose tissue (**A**) and heart (**B**) were taken from one fed (■) and one fasted (△) rat. Increasing amounts of the homogenates were incubated as described in the Materials and methods section. Data points are means of three dilutions of each sample.

rat post-heparin plasma. With tissue homogenates, sample volumes of up to 25 μl gave a linear response (Figure 3). With larger volumes the curves tended to bend, possibly due to saturation. Control experiments showed that the bending was not due to inhibition by the sample buffer. In the following experiments we kept the sample volume below 25 μl. The within- and between-assay variance for the ELISA was determined by using an adipose-tissue extract. The mean within-assay covariance was 5 % and the inter-assay covariance was 20 %.

LPL activity and mass in different rat tissues

To assess the specific activity of LPL in different rat tissues, we fasted five rats overnight and assayed the tissue samples for LPL activity and mass (Table 1). Adipose tissue and heart had the highest amounts of LPL per g of tissue. Soleus muscle, a predominantly red-fibre muscle [27], had almost as high an LPL mass as the heart. The spleen and the lungs also had considerable amounts of LPL protein. Vastus medialis muscle, a predominantly white-fibre muscle [27], had only 20 % of the amount of LPL found in the soleus. The liver and the kidneys had values similar to those found in vastus medialis, whereas the brain had low amounts of LPL mass. Several of the tissues had a high and similar LPL specific activity (heart, soleus, lung, brain), whereas others had significantly lower specific activity (adipose tissue, kidney, liver).

Nutritional effects on LPL in heart and adipose tissue

Table 2 shows LPL activity, mass and specific activity in adipose tissue and heart from five fed and five fasted rats. After an overnight fast the activity in adipose tissue was decreased by 70–80 %, whereas the immunoreactive mass was decreased by only 20–40 %. The specific activity in adipose tissue from the fasted animals was similar to that in Table 1, 0.18 and 0.21 m-unit/ng respectively. In the fed animals, the LPL specific activity had increased to 0.49 m-unit/ng and was now as high as in the group of tissues with high specific activity in Table 1, i.e. heart,

Table 1 LPL activity, mass and specific activity in different rat tissues

Five rats were fasted overnight and killed between 08:00 and 09:00 h. Tissues were collected, prepared and assayed for LPL activity and mass as described in the Materials and methods section. Data for activity are means of duplicate samples, and data for mass are means of three dilutions of the sample. Data are expressed as mean ± S.E.M. ($n = 5$).

	LPL		
Tissue	Activity (m-units/g of tissue)	Mass (ng/g of tissue)	Specific activity (m-unit/ng of enzyme)
Adipose tissue	858 ± 49	4170 ± 230	0.21 ± 0.004
Heart	2251 ± 65	4757 ± 161	0.47 ± 0.013
Soleus	1648 ± 22	3519 ± 88	0.47 ± 0.016
Vastus medialis	196 ± 15	603 ± 53	0.33 ± 0.011
Kidney	91 ± 5	482 ± 33	0.19 ± 0.012
Spleen	613 ± 27	2133 ± 54	0.29 ± 0.015
Lung	851 ± 91	1677 ± 83	0.51 ± 0.029
Brain	54 ± 5	117 ± 10	0.47 ± 0.029
Liver	146 ± 8	778 ± 34	0.19 ± 0.017

Table 2 LPL activity, mass and specific activity: effect of fasting

One group of five rats was fasted overnight, while another group was fed. LPL activity and mass were assayed immediately, as described in the Materials and methods section. Data for activity are means of duplicate samples, and data for mass are means of three dilutions of the sample.

		LPL		
Tissue	Rats	Activity (m-units/g of tissue)	Mass (ng/g of tissue)	Specific activity (m-unit/ng of enzyme)
Adipose tissue	Fed	1863 ± 123	3684 ± 120	0.49 ± 0.004
	Fasted	390 ± 11	2303 ± 218	0.18 ± 0.01
Heart	Fed	1103 ± 110	2516 ± 115	0.48 ± 0.06
	Fasted	1385 ± 124	3035 ± 83	0.45 ± 0.03

soleus, lung and brain. In contrast, no significant differences in either activity or mass occurred in the heart on feeding–fasting.

Relationships between active and inactive forms of LPL in the tissues

To study this, tissues were homogenized in buffer A but without heparin, and separated by chromatography on heparin–agarose columns (Figure 4). Control experiments showed that the amounts of LPL activity and mass extracted without heparin were similar to the amounts extracted with heparin. When the column was eluted by a salt gradient, a peak of inactive LPL was eluted at around 0.6 M NaCl and a peak of active LPL was eluted at 1.0 M NaCl (Figure 4). In adipose tissue from fed rats, LPL mass in the second peak, representing active LPL, was about twice as large as in the first peak, representing inactive LPL. In the fasted state, the relationship was reversed. Hence, the peak ratio (inactive LPL/active LPL) increased from 0.46 ± 0.04 (mean ± S.E.M., $n = 5$) to 2.34 ± 0.13 ($n = 6$). In the heart there were no significant differences in the elution patterns [Figures 4C and 4D; peak ratios 0.44 ± 0.08 ($n = 5$) and 0.43 ± 0.12 ($n = 5$) in fed and fasted, respectively].

DISCUSSION

Earlier studies had shown that the specific activity of LPL differs between different tissues, and changes with physiological circumstances. This could be a result of an altered catalytic efficacy of the LPL molecules or of a changing mixture of active and inactive forms. In the present study we show that there are both active and inactive forms of LPL in rat tissues. In light of previous studies, this is not surprising. Active LPL is a metastable dimer that has a built-in mechanism to self-destruct through dissociation into monomers prone to undergo an irreversible change in conformation [8]. Several cell-culture studies indicate that the main portion of LPL, through a default pathway, is processed into the dimeric, active, form which is secreted from the cells. This process may be responsible for the preponderance of active LPL that we found in the heart, that gives a specific activity of around 0.5 m-unit/ng and which we found also in several other tissues. If this is considered as the default state, we need an explanation for the lower specific activity that we found in adipose tissue after fasting.

We found relatively high amounts of LPL protein, but low LPL activity, in the liver. The LPL activity reported here may be somewhat overestimated, since there is a much larger amount of hepatic lipase in the liver. Hepatic lipase activity was suppressed by preincubation of the liver homogenate with antibodies, but the suppression may not be absolutely complete. Anyhow, our data clearly demonstrate that most of the LPL in liver is inactive. In the adult rat, the liver does not synthesize LPL [28]. Earlier studies have shown that the liver takes up LPL from blood [29]. Then the enzyme loses its catalytic activity through a conformational change [12] and is ultimately degraded. Our present results are in accord with the view that the liver is a site for LPL catabolism rather than LPL production.

The activity of LPL in the soleus muscle was almost as high as in the heart, whereas the activity in the vastus medialis muscle

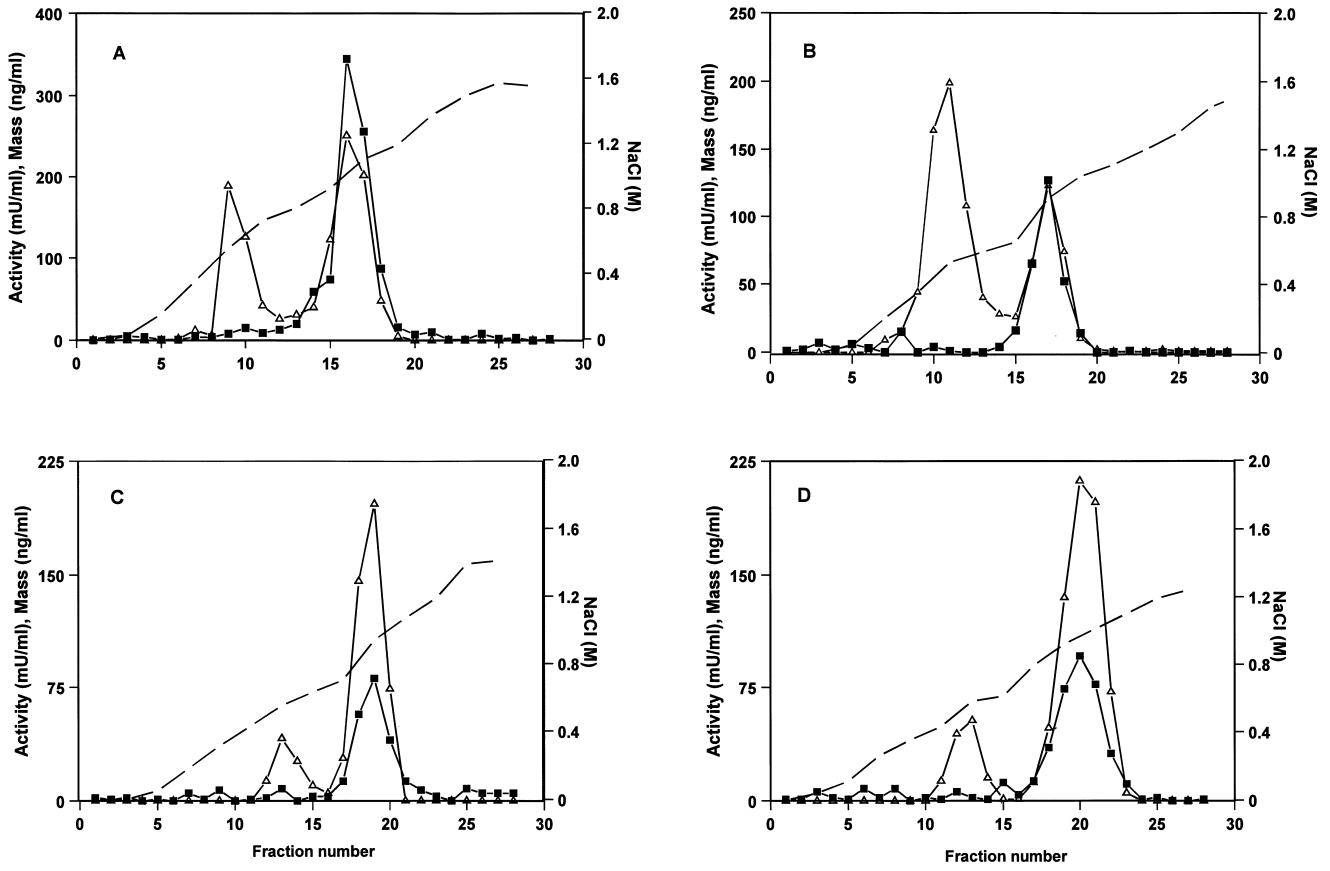

Figure 4 Separation of inactive and active forms of LPL by heparin–agarose chromatography of tissue homogenates

Profiles for adipose tissue are shown in **A** (fed) and **B** (fasted). Profiles for heart are shown in **C** (fed) and **D** (fasted). LPL immunoreactivity (△) was eluted in two peaks, of which only the second was associated with LPL activity. The salt gradient is shown as a broken line. Abbreviation: mU, m-units.

was low. These results are in accord with the results of Tan et al. [27], who concluded that muscles with predominantly red fibres have much higher LPL activity than do muscles with predominantly white fibres. This is logical, since red muscles use more fatty acids for energy production.

The high levels of LPL activity and mass in the spleen and lungs probably originate in the macrophages in these tissues [30,31]. Camps et al. [32], in a study using hybridization *in situ* and immunolocalization, found LPL to be expressed in significant amounts in spleen and lung. In the brain we found low levels of LPL. Previous studies [33,34] revealed the presence of LPL around specific neurons in the brain, and Ben-Zeev et al. [35] found that LPL is synthesized and nutritionally regulated in the adult rat hippocampus. The role of LPL in the brain has not been explained, but our data show that LPL in this tissue is mainly active, i.e. has a high specific activity.

Recent studies have shown that there are several instances of clear separation between changes in the rate of synthesis and the activity of LPL. In a study on developing rats, Tavangar et al. [2] found that LPL activity and mass in heart increased during late gestation and then declined, without any changes in LPL mRNA. In another study by the same group, LPL activity decreased much more than LPL mRNA and mass in adipose tissue from streptozotozin-diabetic rats [5]. Bessesen et al. [36] studied the regulation of adipose-tissue LPL in lean, obese and reduced-obese Zucker rats, and found a small increase in LPL mRNA in obese relative to lean rats and a further increase in reduced-obese rats (reduced-obese rats were given a hypocaloric diet designed to give a 20–25 % weight loss relative to obese rats fed *ad lib.*). These differences could not be correlated with the larger changes that occurred in LPL activity. Semb and Olivecrona [37] found that the changes in levels of adipose-tissue LPL mRNA and activity during fasting in guinea pigs do not occur in parallel. In all, these studies reveal that there are several instances where LPL is regulated post-translationally. Our data indicate that the active form of LPL in adipose tissue has similar specific activity at different nutritional states, but the proportion of inactive LPL changes. The implication is that in the fasted state some newly synthesized LPL does not become active, or loses its activity quickly. This is in concert with the results from Doolittle et al. [3], who found a decrease in LPL activity on fasting for 12 h, but no change in enzyme mass, yielding a decrease in specific activity. Furthermore, they found a shift in the glycosylation pattern of the enzyme, suggesting a change in the intracellular processing and transport. We found a small but significant decrease in adipose-tissue LPL mass during fasting, amounting to 20–40 %. We did not find any changes in LPL activity and mass in the heart, whereas Doolittle et al. [3] found a 2.3-fold increase in heart LPL activity on fasting. These differences could be due to differences in the nutritional protocols. Doolittle et al. [3] achieved a fed state by refeeding of 15 % glucose after a 24 h fast, and a fasted state by food deprivation for 12 h. In our study we

used a 16 h fast to designate a fasted state, and the normal feeding pattern (after overnight feeding on a standard laboratory chow) to designate the fed state. Semb and Olivecrona [37] found that LPL activity in guinea-pig adipose tissue decreased by a factor of 10 on fasting, whereas the levels of LPL mRNA only decreased by a factor of 2. Altogether, these studies show that there is a tissue-specific mechanism to suppress LPL activity in adipose tissue during fasting. The mechanism is not clear, but it is of interest to note the many studies on the role of glycosylation for LPL to become functional. The enzyme needs to be N-glycosylated to become dimeric and active [9,38,39]. The crucial step appears to be removal of terminal glucose residues from the glycan chains [39,40]. The genetic defect cld/cld (for combined lipase deficiency) in mice may relate to this step [41,42]. In these mice, inactive LPL is retained in a high-mannose form within the endoplasmic reticulum.

Ben-Zeev et al. [43] have shown that regulation of heart and adipose-tissue LPL activity are under independent genetic control. In the present study we found no significant changes in heart LPL activity, mass or mass ratio on fasting. Earlier studies as summarized by Borensztajn [44] show that functional LPL in the heart changes with the nutritional state. The amount of LPL that can be rapidly released by heparin, i.e. the functional pool of LPL at the endothelium, increases several-fold on fasting, but the total activity does not change much. Hence the regulation of heart LPL may relate to its binding to the endothelium, rather than to local rates of production or processing [45].

The new aspect in our study is that we have separated inactive and active forms of the enzyme, and we can thus establish that the change in adipose-tissue LPL during fasting is due to a decrease of the relative amount of the active form. The results point to a mechanism in adipose tissue that suppresses LPL activity during fasting, even though the enzyme continues to be synthesized. This would redirect lipid transport to other tissues, but the tissue would be able to recruit active LPL quickly when food becomes available.

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