Regulation of lipoprotein lipase activity and mRNA in the mammary gland of the lactating mouse

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We examined the effects of reproductive stage and fasting on lipoprotein lipase (LPL) activity and mRNA in the mouse mammary gland. Heparin-releasable and cell-associated LPL activity rose immediately after birth, followed 1–2 days later by an increase in LPL mRNA. Fasting decreased LPL activity in the mammary gland at all reproductive stages. During lactation, both milk and heparin-releasable LPL were substantially decreased by an overnight fast, whereas cell-associated LPL was less affected and LPL mRNA did not change. These studies

indicate that the extracellular, heparin-releasable, fraction of mammary LPL activity responds most rapidly to alterations in physiological state, usually accompanied by smaller changes in cellular enzyme activity. Changes in the level of LPL mRNA were seen only during the transition from pregnancy to lactation, and these tended to follow, rather than precede, changes in enzyme activity. We conclude that in the mammary gland as in adipose tissue, LPL is regulated primarily at the translational and post-translational level.

INTRODUCTION

The enzyme lipoprotein lipase (LPL) resides in the capillary lumen in many tissues, most notably adipose tissue, muscle, including heart muscle, and the lactating mammary gland. Its function is to hydrolyse triacylglycerols, making their component fatty acids available for cellular metabolism. The enzyme is synthesized not by endothelial cells [1], but by parenchymal cells in adipose tissue and muscle [2]. In the lactating mammary gland, the enzyme appears to be synthesized by interstitial cells, presumably adipocytes, and transported to the interstitial space where immunocytochemical analysis reveals it to be present in very high concentration [3]. The milk of most species contains high LPL activity, and the milk enzyme has served as the model for many studies of the properties of the enzyme [4]. In a classic study, Hamosh and colleagues [5] showed that the enzyme is elevated in mammary gland and depressed in adipose tissue during lactation (see also [6–8]), allowing shunting of lipids away from adipose tissue to milk synthesis.

Tissue LPL activity depends on the physiological state of the animal, changing in response to feeding/fasting, cold adaptation, exercise and tumours [9]. During fasting, adipose-tissue LPL activity undergoes a profound decrease in many species [2, 10–13]. In guinea pigs both changes in mRNA and changes in transport and turnover of the enzyme were shown to be involved [10]. Refeeding with glucose alone rapidly reversed these changes [14]. Muscle LPL, on the other hand, tends to increase during fasting [15], an effect that has been attributed by some to β -adrenergic stimulation [16]. Studies on the effect of physiological state on LPL activity in the lactating gland are fewer: a 24 h fast, decreased prolactin, sealing of the teats and removal of the pups for 24 h, all of which decrease milk production, were shown to decrease LPL activity in the lactating mammary gland of the rat [17, 18]. More recently, an injection of an antibody against rat growth hormone in combination with bromocryptine, an inhibitor of prolactin secretion, was shown to inhibit both mammary and adipose-tissue lipoprotein lipase in the same animal [19]. However, a detailed study of the effects of physiological

state on LPL activity and correlation of the activity with mRNA expression in the mammary gland is lacking.

These considerations led us to undertake a study in mice. This species was used because of the availability of systems *in vitro* for future studies of detailed molecular mechanisms. We examined the effect of reproductive state and the fed–fasting transition on LPL activity and mRNA in the mammary gland. We examined LPL activity in milk, as well as the heparin-releasable and cell-associated activity in mammary tissue. These experiments show that mammary LPL activity, like the LPL activity of adipose tissue [10, 20], is subject to regulation at both the mRNA and the post-translational levels, but post-translational regulation appears to dominate both during the transition from pregnancy to lactation and in the fasting state.

METHODS

Animals and tissues

A breeding colony of CD1 mice (Charles River) was maintained in the approved animal facility of the University of Colorado Health Science Center. Lactating and pregnant animals were fed on breeder chow, consisting of 14% protein and 6% fat with metabolizable energy of 13.8 kJ (3.3 kcal)/g. Virgin animals were fed chow consisting of 22% protein and 5% fat with a metabolizable energy of 14.6 kJ (3.5 kcal)/g. The number of pups was standardized to 9–11 within 2 days of birth. When only tissues were to be obtained, animals were allowed to suckle until they were killed, which was done by cervical dislocation, and the appropriate tissues were dissected immediately. Parametrial adipose tissue was dissected away from the uterus, and ventricular muscle was dissected for cardiac LPL. For milk samples from lactating mice, pups were removed 1–2 h before the experiment. Animals were anaesthetized with an intraperitoneal injection of pentobarbital (0.65 mg/10 g body wt.) and 0.1 unit of oxytocin was given by intracardiac injection. Milk was removed as completely as possible by syringe from a slit at the base of each teat. After milking, the fourth and fifth mammary glands were removed for further analysis. Immediately after dissection,

Abbreviations used: LPL, lipoprotein lipase; KRP, Krebs–Ringer phosphate.

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several 100 mg pieces of mammary gland were rapidly frozen in a methanol/solid- CO_2 bath and stored at -70°C for later mRNA extraction. The remainder of the tissue was placed in ice-cold Krebs–Ringer phosphate (KRP; 0.13 M NaCl, 5.14 mM KCl, 1.7 mM CaCl_2 , 1.3 mM MgSO_4 , 1.3 mM KH_2PO_4 and 10 mM Na_2HPO_4 , pH 7.4) and processed for LPL assay as described below. For studies of feeding and fasting, food was removed from cages at 22:00 h. At 08:00 h the following day, animals were immediately killed (fasting) or allowed access to food for 2 h (refed). Controls were allowed to feed *ad libitum*. All protocols were approved by the Animal Care Committee of the University of Colorado Health Sciences Center.

LPL assay

Tissue

The fourth and fifth mammary glands were dissected and placed in ice-cold KRP as described above. Unless otherwise stated, tissues were minced with sharp scissors into pieces less than 1 mm³ while in this solution to allow any residual milk to diffuse from the tissue. After 15–30 min, duplicate or triplicate tissue samples (about 45 mg) were incubated for 45 min at 37°C in 400 μl of KRP containing 3.9 $\mu\text{g}/\text{ml}$ sodium heparin (Fischer Scientific, purified grade) to release interstitial enzyme. This concentration of heparin was shown to produce maximal LPL release from mouse mammary gland (results not shown). LPL released into this medium was considered to represent extracellular lipase; however, it is possible that it also includes some secreted enzyme as well. In some preliminary experiments, protease inhibitors (phenylmethanesulphonyl fluoride, 150 $\mu\text{g}/\text{ml}$; leupeptin, 1 $\mu\text{g}/\text{ml}$; or iodoacetamide, 150 $\mu\text{g}/\text{ml}$) were tested in these solutions; except for phenylmethanesulphonyl fluoride, which was slightly inhibitory in the LPL assay itself, addition of protease inhibitors had no consistent effect on LPL recovery. Duplicate 100 μl portions of the supernatant were assayed for LPL activity by measuring the hydrolysis of [^{14}C]triolein in the presence of serum, which contains the essential activator of LPL, apolipoprotein CII, as described previously [3]. To assay intracellular LPL, the remaining KRP/heparin was removed from the tissue pieces, which were then homogenized in 1 ml of a detergent solution (0.2 M Tris, pH 8.2 with 0.7% sucrose, 1% fatty-acid-free BSA, 0.5% deoxycholate, 0.02% Nonidet P-40 and 0.01% heparin), followed by addition of 2.5 ml of the same solution without deoxycholate, Nonidet P-40 and heparin [21]. After centrifugation (1000 g, 15 min, 4°C), duplicate 100 μl samples of the infranatant were assayed for LPL activity as above. Within a single experiment, all samples were assayed with the same lot of freshly prepared substrate to avoid intra-assay variation. In some experiments, hydrolysis in the absence of serum was assayed to permit a correction for non-specific lipases to be made. In milk and extracellular fractions, LPL was responsible for at least 90% of lipase activity. In cell-associated fractions it made up at least 75% of the total lipase activity.

Milk

Mouse milk was diluted 1:500 with KRP containing 4 $\mu\text{g}/\text{ml}$ heparin, and a 100 μl sample was assayed for the ability to hydrolyse [^{14}C]triolein as described above.

DNA assay

DNA content was measured in homogenates of weighed tissue samples by using a fluorescence DNA assay developed by

Labarca and Paigen [22]. Tissues were homogenized in 0.05 M $\text{NaH}_2\text{PO}_4/2$ M NaCl/2.0 mM EDTA, adjusted to pH 7.4 with NaOH; 10 μl samples were removed, diluted to 3 ml, and 16 μl of bisbenzimidazole (20 $\mu\text{g}/\text{ml}$) was added. After incubation at 25°C for 15 min, the fluorescence was measured at excitation and emission wavelengths of 345 and 492 nm respectively.

RNA isolation and measurement of specific mRNAs

Total cellular RNA was prepared from weighed and frozen tissue fragments by the guanidinium thiocyanate method of Chirgwin et al. [23] and collected by precipitation with propan-2-ol followed by ethanol. After spectrophotometric quantification, 20 μg of each RNA sample was electrophoresed on an agarose gel containing ethidium bromide. The 18 S and 28 S rRNA bands were revealed to ensure RNA integrity, and the RNA was transferred to nitrocellulose, baked for 2 h and hybridized by standard high-stringency methods [24] to DNA probes for LPL, actin, mouse β -casein and mouse histone 3.3 labelled with [^{32}P]CTP by nick translation (Boehringer Mannheim Corp., Indianapolis, IN, U.S.A.). Unhybridized probe was removed by washing twice with $2 \times \text{SSC}$ (17.5 g of NaCl, 8.82 g of sodium citrate, pH 7.0) and once with $0.1 \times \text{SSC}$ containing 0.1% SDS. The probes used were, for LPL, an 872 bp segment of rat LPL cDNA containing 422 bp of coding sequence and 450 bp of 3' untranslated region [25]. The high degree of conservation of LPL

Figure 1 (a) Autoradiograms showing hybridization of probes for casein, LPL, actin and mouse histone H3.3 to poly(A)⁺ RNA from the mammary glands of mice at days 14, 17 and 21 of pregnancy and days 1 and 2 of lactation; (b) ratios of actin (■), LPL (○) and casein (●) mRNA to mouse histone H3.3 mRNA as a function of reproductive stage

In (a) the difference in density between the various species of mRNA reflects probe radioactivity and exposure time; it is not related to the relative amounts of the various RNA species in the tissue. In (b), day 0 is defined as the final day of pregnancy (day 21); mice were born sometime during this day. Binding of labelled probe was quantified directly with the AMBIS radioanalytic imaging system as described in the Methods section.

between species at the DNA level made it possible to use the rat cDNA to probe for mouse mRNA. For mouse H3.3 histone a 510 bp segment of cDNA, kindly provided by William Marzluff of Florida State University, was used. This is a histone variant that is not developmentally regulated and has a poly(A) tail [26]. Mouse β -actin cDNA is described in [25]. The mouse β -casein probe was provided by Jeffrey Rosen of Baylor College of Medicine, and was the clone designated B in [27]. Because the signal was low from the pregnant gland, for quantification of the effect of the developmental stage of the mammary gland on LPL mRNA levels poly(A)⁺ mRNA was prepared before chromatography and hybridization. We used the polyATtract mRNA isolation system from Promega and recovered about 1.5 % of the total RNA as mRNA. For Northern blotting a constant amount of RNA was run in each lane (ranging between 1 and 3 μ g from experiment to experiment). Binding of labelled probe was quantified in these experiments by direct counting of the radioactivity associated with each band, by using an AMBIS radioanalytic imaging system in the grid-quantification mode. This scanner contains 952 radioactivity-detection elements in a grid design along with a computer-driven scanner that allows counting of 70000 points on a 20 cm $\times$ 20 cm two-dimensional surface. Counting time was set to accumulate at least 4000 counts per band; background counts from an adjacent unlabelled area of the surface were subtracted.

Figure 1(a) shows autoradiographic bands produced by hybridization of all four probes to poly(A)⁺ RNA from days 14, 17 and 21 of pregnancy and days 1 and 2 of lactation, by using pooled poly(A)⁺ RNA samples from three mice. The gels from which these autoradiographs were developed were counted on the AMBIS system, and the ratio between counts on each day and the counts on day 14 of pregnancy was calculated. The ratio of actin to histone mRNA (Figure 1b) was approx. 1 over the period studied, whereas the ratio of casein to histone increased markedly on the day of birth. The ratio of LPL mRNA to histone mRNA increased substantially only on day 2. These results verified that histone H3.3 mRNA was an adequate loading standard and cellular reference for the estimation of specific changes in LPL mRNA; this probe was used previously in this way for the developmental analysis of casein mRNA [27]. This method was used for subsequent analysis of developmental changes in LPL mRNA reported below.

The effect of fasting on LPL mRNA levels was measured only in lactating mice. In this case total RNA was electrophoresed, blotted to nitrocellulose and hybridized to histone and LPL probes as described above. After hybridization the nitrocellulose was washed and exposed to X-ray film, and the intensity of the signal was quantified by scanning densitometry. Care was taken to avoid saturating densities of signal, and re-exposures were performed if necessary.

RESULTS

Effect of reproductive stage and fasting on LPL in mouse tissues

In an initial experiment, we determined the effect of reproductive stage and fasting on the extracellular activity on LPL in the mammary gland, adipose tissue and heart (Table 1). In the mammary gland, as has been amply demonstrated in other species, LPL activity was low in virgin animals, progressively increasing during pregnancy and lactation. Adipose-tissue LPL increased during pregnancy, concomitant with the increase in adipose-tissue stores. It then decreased as lactation progressed, reflecting the vastly diminished adipose-tissue stores at the height of lactation in mice. In fact, it was not possible to measure adipose-tissue LPL activity on day 10 of lactation in this strain of mouse, because there was no apparent adipose tissue remaining in the animal. The LPL activity of cardiac muscle was maintained during pregnancy and early lactation, but increased markedly at day 10 of lactation, probably reflecting the increased cardiac output necessary to maintain nutrient uptake from the gut and transport to the mammary gland for milk synthesis [28]. Overnight fasting had a profound effect on LPL activity in both mammary gland and adipose tissue at all reproductive stages. The effects of overnight fasting on cardiac LPL were less consistent.

These experiments established that LPL activity in mammary and adipose tissue from mice behaves in a manner similar to LPL activity in the same tissues from other species [5]. They also demonstrated the similarity between mammary gland and adipose tissue in their response to an overnight fast. In the next set of experiments we compared the LPL activity in the mammary gland with that of mouse milk, and determined that it was possible experimentally to separate interstitial LPL activity from LPL activity of the milk contained within the tissue.

Table 1 Effect of reproductive stage on heparin-releasable LPL activity

See the text for methods. Values are means $\pm$ S.E.M. for three mice in each state. *Significantly different from virgin ($P < 0.01$); †significantly different from fed *ad lib.* ($P < 0.01$).

Tissue	Reproductive stage	LPL activity (nmol of fatty acid/min per mg of DNA)		
		Fed <i>ad lib.</i>	Fasted	Refed
Mammary gland	Virgin	4.7 $\pm$ 2.4	3.4 $\pm$ 0.1	3.4 $\pm$ 0.2
	Pregnant (14 days)	20.1 $\pm$ 2.6*	3.1 $\pm$ 0.6†	16.0 $\pm$ 0.9
	Lactating (4 days)	55.7 $\pm$ 4.7*	16.1 $\pm$ 6.0†	18.0 $\pm$ 2.2†
	Lactating (10 days)	80.8 $\pm$ 1.6*	3.0 $\pm$ 0.6†	16.1 $\pm$ 1.0†
Adipose tissue	Virgin	78.1 $\pm$ 5.3	19.2 $\pm$ 2.8†	36.1 $\pm$ 3.0†
	Pregnant (14 days)	115.6 $\pm$ 28.3	19.2 $\pm$ 2.8†	109.7 $\pm$ 20.6
	Lactating (4 days)	51.2 $\pm$ 25.9	17.3 $\pm$ 13.6†	74.9 $\pm$ 11.2
	Lactating (10 days)	—	—	—
Cardiac muscle	Virgin	36.9 $\pm$ 1.3	75.6 $\pm$ 6.2†	26.4 $\pm$ 1.9†
	Pregnant (14 days)	48.7 $\pm$ 4.4*	57.4 $\pm$ 3.8	62.7 $\pm$ 6.5
	Lactating (4 days)	33.6 $\pm$ 2.6	19.5 $\pm$ 2.8†	13.6 $\pm$ 0.9
	Lactating (10 days)	66.5 $\pm$ 10.1*	57.4 $\pm$ 3.8	62.7 $\pm$ 0.4

Figure 2 Effect of abrupt weaning on LPL activity of milk and tissue

Litters were removed from 10-day-lactating mice at intervals before killing. Milk was collected and assayed as described. Heparin-releasable tissue LPL (n-equiv. of fatty acid/min per g of tissue or milk) was assayed after 45 min incubation at 37 °C.

Relation between milk and tissue LPL activity

In lactation, the mammary gland gains an appreciable milk compartment, leading us to ask to what extent changes in milk LPL activity reflect changes in tissue LPL activity. Pup removal had a dramatic effect on the LPL activity of both milk and mammary gland (Figure 2). For this experiment pups were removed from 10-day-lactating mice, and milk and tissue samples were taken at various times. In this case the glands were all quite milky, even after extraction of milk from the gland *in situ* with oxytocin. Milk and mammary activity were high 3 h after pup removal, but both fell in parallel to nearly zero by 10 h in the unmilked state. This experiment also illustrated the very high activity of LPL in mouse milk, at least 10 times the tissue values (the latter are expressed per g of tissue for comparison with milk, rather than per mg of DNA as in Table 1 and Figure 4).

Data similar to those in Figure 2 led us to question whether it was possible to measure interstitial LPL activity apart from the milk contained within the tissue. We examined the flux of LPL into Ringer solution at 0 °C and 37 °C (Figure 3), using a technique which we had previously used to examine Ca^{2+} [29] and lactose [30] fluxes. A strip of mammary tissue about 1 mm in diameter and 1–2 cm long was cut from the fourth gland and tied with a string to allow it to be moved from one test tube to another at precisely timed intervals. The tissue was incubated in a series of five tubes containing Ringer solution and agitated in either an ice bath or a 37 °C water bath. At 30 min the tissue was moved to Ringer containing heparin, and the incubation continued for another 45 min at 37 °C. The amount of activity in tubes 5–1 was progressively summed backwards to obtain the percentage of activity remaining in the rapidly exchanging fraction at each time point. The data were fitted to the equation for efflux from a cylinder [29]:

$$\frac{C_t}{C_0} = 4 \sum_{n=1}^{\infty} \frac{1}{\nu_n^2} \exp(\nu_n^2 D \lambda^2 t / r^2) \quad (1)$$

where the ratio of the concentration at time t (C_t) to the initial concentration (C_0) is plotted as a function of time. The roots of the Bessel function are given by ν , D is the diffusion coefficient, λ is a tissue tortuosity factor that has a value of about 2 in mammary tissue [29], t is the time, and r the radius of the

Figure 3 Loss of LPL from strips of mammary gland

Duplicate strips of lactating mammary gland were tied with string and moved through a series of five tubes containing 1 ml of KRP each placed in a shaking ice bath (●, ○) or a 37 °C water bath (◆, ◇). At 30 min, the tissues were placed in KRP containing 3.9 µg/ml heparin for 45 min at 37 °C, and released LPL activity for each sample was plotted as indicated by the arrow. LPL activity in the solutions was assayed as described in the text, and the amounts in the wash solutions summed as described to produce the efflux curves. To allow comparison of the results of two different experiments, all results are plotted as the percentage of the total activity in the wash solutions. Lines are theoretical according to eqn. (1). Black and white symbols represent tissues from different animals.

cylinder. At long times only the first term in eqn. (1) contributes significantly, and the diffusion coefficient can be calculated from the slope of the curve. The coefficients calculated were $1.2 \times 10^6 \text{ cm}^2/\text{s}$ at 37 °C and $0.5 \times 10^6 \text{ cm}^2/\text{s}$ at 0 °C. The ratio of the two coefficients was 0.42, close to the ratio of 0.44 calculated for the effect of temperature on the loss of Ca^{2+} from the extracellular space of similar mammary strips [29]. The amount of LPL recovered from the wash phase in the 37 °C experiment was 44 nmol/min per g of tissue, equal to the activity found in 54 µl of mouse milk. This is equivalent to a milk space in the tissue of 5.4%, similar to the milk space of 6.4% found in previous studies of lactose flux from mouse mammary gland [30].

Additional LPL was released during the 37 °C incubation with heparin (Figure 3b). In the case of the 37 °C wash, the amount released was about 60% of the total activity released into the wash. After the 0 °C wash the amount released into the heparin solution was about 200% of the total released during the wash phase. We consistently found that less total LPL was recovered when the wash was carried out at 37 °C, and surmise that the 0 °C treatment washes out proteases released by tissue damage during the dissection that normally decrease the LPL activity measured during the subsequent hydrolysis assay.

These findings formed the basis of the technique used to examine interstitial LPL in the mammary gland. Instead of strips, we diced the gland into small particles approximating to spheres with a maximum diameter of 1.0 mm. With this shape the rate of efflux is a function of the square of the radius, rather than the radius as for the cylinder described above [31]. Using the diffusion coefficient calculated above, we calculate [31] that the half-time of exchange for LPL would be less than 3 min at 0 °C. This means that no more than 3% of the milk originally present in the tissue should be present at 15 min. Since the milk space of the gland is about 6% at the start of the experiment and the milk contains about 10-fold higher LPL activity than the tissue, not more than 2% of the LPL activity recovered in the KRP/heparin fraction originates from residual milk in the tissue.

Transition from pregnancy to lactation

Heparin-releasable and cell-associated LPL activity were measured in mammary tissues taken from day 14 of pregnancy until

Figure 4 Changes in LPL enzyme activity and mRNA levels during mammary-gland development

Tissue LPL activity was assayed in the heparin-releasable (a) and intracellular (b) fractions. Each point represents the mean $\pm$ S.E.M. of values from the number of animals given in (a). (c) Ratio of LPL mRNA to histone H3.3 mRNA normalized to day 14 of pregnancy as determined by Northern blots quantified as in Figure 1. The number of animals killed at each point is shown on the Figure.

day 9 of lactation and correlated with the corresponding mRNA levels (Figure 4). There was a 20-fold increase in heparin-releasable LPL activity between birth and day 1 of lactation, followed by a more or less continuous rise in activity to day 9 (Figure 4a). Cell-associated LPL activity increased rapidly on both days 1 and 2 of lactation, levelling out thereafter (Figure 4b). Relative to histone H3.3, LPL mRNA rose insignificantly over the period of parturition, but doubled between days 2 and 4 of lactation (Figure 4c). These observations suggest that changes in LPL mRNA levels follow changes in enzyme activity during mammary development, rather than initiating them.

Effects of feeding and fasting

Since developmental changes in LPL activity in the mammary gland did not appear to be driven by changes in LPL mRNA, we designed experiments to determine whether the effects of fasting were correlated with changes in mRNA. Table 2 shows the LPL activity of the milk and the various tissue fractions. Milk LPL

Table 2 Effect of fasting and refeeding on LPL activity in milk, and on LPL activity and mRNA in mammary gland, from lactating mice

Values are means $\pm$ S.E.M. for three mice in each state. Abbreviation: FA, fatty acid. *Significantly different from fed *ad lib.* ($P < 0.01$).

	Fed <i>ad lib.</i>	Fasted	Refed
Milk LPL activity (n-equiv. of FA/min per ml)	3670 $\pm$ 610	560 $\pm$ 200*	1480 $\pm$ 488*
LPL activity in wash solution (n-equiv. of FA/min per mg of DNA)	113.7 $\pm$ 30.8	6.4 $\pm$ 2.3*	16.7 $\pm$ 4.4*
LPL activity in KRP-heparin solution (n-equiv. of FA/min per mg of DNA)	106.6 $\pm$ 16.5	31.9 $\pm$ 4.2*	61.4 $\pm$ 6.0*
Cell-associated LPL activity (n-equiv. of FA/min per mg of DNA)	313.7 $\pm$ 41.4	208.8 $\pm$ 31.1	277.6 $\pm$ 42.0
LPL mRNA (ratio LPL/histone)	3.2 $\pm$ 0.4	2.9 $\pm$ 0.2	2.9 $\pm$ 0.2

activity was decreased by 85% after the 10 h night-time fast; refeeding for 2 h began to restore milk LPL activity, but not to the levels observed in the fed animal. The activity in the solution in which the tissue was minced, mainly representing milk lost from the gland, was decreased by about 95% by fasting and showed only slow recovery after 2 h of refeeding. The LPL activity in the KRP/heparin solution was decreased by about 70% by fasting, but the cell-associated activity was decreased by only about 30%. These observations suggest that the major effect of fasting on the LPL activity of mouse mammary gland is exerted on the milk. Whether the milk activity reflects LPL activity at its site of action in the capillary cannot be determined from the present experiments. The smaller decrease in intracellular LPL observed, along with the observation that the levels of mRNA in the tissue are changed insignificantly by fasting (Table 2), suggests that the major effect is post-translational.

DISCUSSION

LPL activity in mouse milk

In these experiments we have demonstrated the extraordinarily high activity of LPL in mouse milk (approx. 4000 n-equiv. of fatty acid/min per ml, compared with activities of 200 n-equiv. of fatty acid/min per ml in human milk [32], 140 n-equiv. of fatty acid/min per ml in rat milk and 1290 n-equiv. of fatty acid/min per ml in bovine milk, as determined in this laboratory). Although the activity in mouse milk is high, immunocytochemical analysis suggests that the highest concentrations of LPL protein in the mouse mammary gland are associated with the adipocyte cytoplasm and alveolar basement membranes [3]. Immunocytochemical staining of LPL was similar in rat and mouse mammary glands (M. C. Neville, unpublished work), suggesting that the low activity of the enzyme in rat milk may be due to loss of the activity, rather than the absence of protein from the milk. Comparison of protein mass with enzyme activity in rat milk is needed to confirm this hypothesis.

In humans, milk LPL activity appeared to parallel the rate of milk production [32,33] suggesting that the demand for milk fat synthesis is, in part, met by increases in LPL activity in the gland. In the mouse a similar effect is seen, particularly prominent during the onset of lactation (Figure 4) and with variations in litter size (M. C. Neville, unpublished work). Conditions such as weaning or fasting elicit parallel changes in LPL activity in the milk and mammary tissue.

Figure 5 Model for LPL transport in the mammary gland

See the text for explanation. Abbreviations: FDA, fat-depleted adipocyte; BM, basement membrane.

Developmental changes in LPL mRNA and enzyme activity

Tavangar et al. [34] examined developmental regulation of LPL activity in rat tissues, noting very little relation between LPL activity, LPL mass and LPL mRNA in heart, epididymal fat-pad or kidney. In the mammary gland, LPL activity in the heparin-releasable and intracellular compartments of the gland decreased somewhat during late pregnancy, accompanied by very little change in the proportion of LPL mRNA (Figure 4). A decrease in mammary LPL activity in rats [5] was postulated to be due to the hormonal changes of late pregnancy, with secondary effects on LPL activity [35]. More simply, however, this decrease may reflect dilution of the enzyme as the gland gears up for the synthesis of milk proteins, a process that begins a few hours *pre partum* in the rat [36]. In the 48 h immediately after birth, the interstitial LPL activity increased more than 10-fold in the mouse gland (Figure 4) and the cell-associated activity tripled. Subsequently the interstitial activity continued to increase, while the cell-associated activity remained relatively stable.

Although the total mRNA in the tissue increased about 2-fold just before birth (results not shown), the proportion of LPL mRNA remained low for at least 24 h after birth, rising to a maximum at 4 days. There was no change in mRNA between the last day of pregnancy and the first day of lactation that could account for the marked increase in enzyme activity at that period. These experiments do not permit us to say whether changes in mRNA are caused by an increased rate of transcription, or a decreased rate of mRNA turnover, or even an increase in the number of LPL-producing cells. The delay between the major increase in enzyme activity and the onset of the increase in mRNA suggests that regulation of enzyme activity involves a major element of translational or post-translational regulation. This result is consistent with the findings by Barber et al. [19] that the decreased mammary LPL activity induced by anti-(rat growth hormone) antiserum and bromocryptine in lactating rats was accompanied by an insignificant change in LPL mRNA.

Effects of nutritional status on LPL activity in the mammary gland

Overnight fasting depressed interstitial LPL activity in the mammary gland at all stages of development (Table 1). During

lactation we found that the enzyme activities in the milk and interstitial compartment were more sensitive to fasting than was activity in the cell-associated fraction (Table 2). This observation, along with the finding that steady-state mRNA levels do not vary significantly between the fed and fasted states, again suggested that the response to fasting is regulated at a post-translational level. Similar inferences have been made in a number of studies of the effect of fasting on adipose tissue LPL [10–13,37]. However, long-term fasting in rats was associated with proportional decreases in both adipose-tissue LPL activity and its mRNA [11].

Model for regulation of LPL activity in the mammary gland

Overall, the results in this paper, along with those in our previous publications [3,32], are consistent with the model shown in Figure 5. In this model, LPL is produced in adipocytes in the interstitial spaces of the mammary gland, a concept for which we have previously presented immunocytochemical and other evidence [3]. The present finding, that effects of fasting on mammary LPL are similar to the effects of fasting on adipocyte LPL, is consistent with this model. The major regulatory features of this model can be understood as follows. (i) In the virgin mouse the underdeveloped gland requires little LPL and enzyme activity is low. (ii) During pregnancy (Figure 5a), moderate tissue levels of LPL facilitate fat storage in both adipocytes and epithelial cells in preparation for lactation. Interstitial and capillary LPL activity respond to feeding and fasting as in adipose tissue: if the model of Bensadoun [38,39] and others [40] is applicable to the lactating mammary gland, during fasting LPL would be recycled to the adipocyte for degradation. (iii) During lactation (Figure 5b), in response to the demand for lipid for milk synthesis, substantial quantities of the enzyme are transported to the capillary lumen, probably by means of heparan sulphate proteoglycan receptors [41–43]. Hydrolysed lipids are shunted away from adipocytes to mammary epithelial cells, resulting in depletion of the fat content of mammary adipocytes [44]. LPL enters the mammary alveolar cells for transport to the milk. (iv) In the fasted state (Figure 5c), LPL transport to the capillaries and mammary alveolar cells is rapidly decreased and the milk activity is rapidly depleted. Because cell-associated enzyme and mRNA are less affected by fasting than interstitial activity (Table 2), it seems likely that LPL protein synthesis continues and that increased internalization

and degradation into the adipocyte account for the decreases in interstitial and milk LPL activity.

This hypothesis raises a number of important questions. (i) What are the signals that up-regulate mammary LPL activity and down-regulate adipose-tissue LPL during lactation? The findings by Barber et al. [19], that an antibody against rat growth hormone increased both adipose-tissue LPL and serum insulin in lactating rats, suggest that growth hormone or insulin or both may play a role in adipose-tissue LPL regulation during lactation. In the mammary gland, anti-(growth hormone) antiserum decreased LPL activity, but only in the presence of bromocryptine to inhibit prolactin release. It seems likely that these hormone effects are indirect, mediated by a local signal dependent on substrate utilization for milk secretion. The possible role of fat depletion in the mammary adipocytes requires investigation in this regard. (ii) How is LPL transported to and across the mammary alveolar cell? Although heparan sulphate proteoglycans have been implicated in LPL transport into capillaries [41–43], the mechanism of transport into mammary cells has not been investigated. (iii) Does milk LPL have a function in the newborn, or is its presence simply indicative of a convenient disposal pathway? These questions and many others remain for future research.

Regardless of the answers to these questions, the results of our investigations indicate that acute changes in LPL are mediated by post-translational events, whereas longer-term responses such as those observed from days 2–4 of lactation may involve changes in mRNA levels. This dual control is obviously beneficial, allowing a rapid response to an acute demand for lipid substrate, with longer-term increases in demand being met by increased message transcription and increased enzyme synthesis.

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