

Distribution and source of lipoprotein lipase in mouse mammary gland

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Abstract During lactation lipoprotein lipase (LPL) is elevated in mammary tissue and depressed in adipose tissue to redirect lipids for incorporation into milk fat. The cellular origin of lipoprotein lipase in mammary tissue is thought to be the mammary epithelial cell which is the predominant cell type noticeable in the lactating gland; however, mammary adipocytes are also present. If lipoprotein lipase is produced by adipocytes in other sites of the body, then the question remains as to why mammary adipocytes have not been shown to produce lipoprotein lipase. In this study we present several lines of evidence that indicate that the mammary adipocyte is a source of LPL in the lactating mammary gland of mice. This evidence includes the absence of extracellular and intracellular lipoprotein lipase activity in two types of primary mammary epithelial cell cultures and a similarity in the changes of lipoprotein lipase activity in genital adipose tissue from nonpregnant mice and lactating mammary tissue to the nutritional state of the animal. Other evidence presented here includes strong localization of lipoprotein lipase protein and messenger RNA by fluorescence immunohistochemistry and in situ hybridization, respectively, to interstitial cells located between epithelial structures. We postulate that these interstitial cells are regressed, lipid-deleted mammary adipocytes. —Jensen, D. R., D. H. Bessesen, J. Etienne, R. H. Eckel, and M. C. Neville. Distribution and source of lipoprotein lipase in mouse mammary gland. *J. Lipid Res.* 1991. 32: 733-742.

Supplementary key words lactation • mammary • epithelial cells • adipocytes • immunohistochemistry • in situ hybridization

During lactation lipid metabolism is regulated in such a way as to direct substrates away from adipose tissue and into milk synthesis (1, 2) resulting in increased lipolysis (3, 4) and decreased fat cell size (5) in adipose stores within and external to the mammary gland. Lipoprotein lipase (LPL) appears to play a major role in this adaptation (6). Several laboratories have shown that the enzyme is elevated in mammary tissue and depressed in adipose tissue (4, 7-10) enabling dietary and stored lipids to be preferentially incorporated into milk fat. The endocrine mechanisms that modulate the reciprocal relationship between mammary and adipose tissue are poorly under-

stood although there is evidence for the involvement of growth hormone (11), prolactin (7, 8, 12), and insulin (13, 14).

LPL, bound to the heparan sulfate proteoglycans in capillary walls (15, 16), hydrolyzes triglycerides in chylomicrons and very low density lipoproteins to fatty acids and monoglycerides (17, 18). In adipose tissue it is clear that the adipocytes are the source of LPL synthesis and secretion (19). The cellular origin of LPL in mammary tissue is unclear. The enzyme has been assumed to be produced by the epithelial cells because LPL activity is high in lactating mammary tissue and epithelial cells are the predominant cell type noticeable by light microscopy (20). The presence of LPL in milk and its association with mammary epithelial cell membranes (21) support this concept (4). Using in situ hybridization, Camps and coworkers (22) have recently reported that LPL mRNA is associated with glandular tissue in the lactating guinea pig.

Although not well studied, it is also possible that another cell type, namely the mammary adipocyte, is an important source of LPL in mammary tissue. Rebuffé-Scrive and coworkers (23) showed that LPL activity is closely associated with adipocytes obtained during breast biopsy in nonpregnant women. We have found that the LPL activity in human milk is subject to regulation by insulin in a manner similar to the LPL activity of adipose tissue (24). Finally, because LPL is produced by adipocytes in other sites in the body, it seems reasonable that mammary adipocytes might have that capacity as well. We have used several approaches to examine this idea more closely.

Abbreviations: LPL, lipoprotein lipase; PBS, phosphate-buffered saline; FA, fatty acid.

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Initially, we compared LPL activity in two types of functional cultures of mammary epithelial cells with LPL activity in adipocyte cultures. When the mammary cultures were found to lack LPL activity, we measured LPL activity released by heparin in lactating mammary gland and genital fat from nonpregnant mice. Finally, immunohistochemistry and *in situ* hybridization for LPL protein and mRNA, respectively, were performed to localize LPL and its synthesis in sections of tissue from lactating mammary gland.

MATERIALS AND METHODS

Animals

CD-1 mice from Charles River were maintained as a breeding colony in the Animal Resource Center of the University of Colorado Health Sciences Center. Animals were killed by cervical dislocation at 14 days pregnancy or 10 days lactation. The fourth and fifth mammary glands were dissected and treated as described below. Animals assigned to the fed group were allowed to feed *ad libitum* throughout the night. Food from mice to be fasted (nonpregnant and lactating) was removed at 10:00 PM prior to experimentation and these mice were killed between 8:00 and 10:00 AM. Refed mice were fasted overnight and then refed *ad libitum* for 2 h prior to being killed. The number of pups was standardized at nine to eleven in each brood and they were allowed to suckle up to the time of killing in all groups. All animals were given water *ad libitum*.

LPL activity and DNA assays

The LPL activity assay is based on the method of Eckel et al. (25). The heparin-releasable LPL activity was measured by incubating the tissue sample 45 min at 37°C in Krebs Ringer phosphate buffer (pH 7.4) containing 3.9 µg/ml sodium heparin. This concentration of heparin was shown to promote maximal LPL release (data not shown). For measurement of intracellular LPL activity after heparin release, the tissues were then homogenized by hand on ice with 200 µl of a detergent solution (0.2 M Tris, pH 8.2, with 0.7% sucrose, 1.0% fatty acid free albumin, 0.5% deoxycholate, 0.02% Nonidet P-40 and heparin and centrifuged (3000 g, 30 min, 4°C) to remove tissue debris (25). Aliquots of either heparin-released material or the middle layer from the supernatant of the homogenate were assayed for LPL as previously described. Substrate was prepared containing [¹⁴C]triolein, triolein, phosphatidylcholine, and pooled serum as a source of apoC-II. A 100-µl aliquot of substrate was incubated with 100 µl of sample for 45 min at 37°C. The reaction was stopped and lipids were solubilized with chloroform-methanol-heptane (26) and bicarbonate buffer (50 mM NaHCO₃, pH 10.5) was added to allow phase separation and partition of fatty acids to the

aqueous phase. After mixing and centrifugation at 2000 g for 20 min at 4°C, the ¹⁴C activity in a 500-µl aliquot was counted.

DNA content for each tissue was determined using a fluorescence DNA assay (27). Briefly, tissues were homogenized in a phosphate-saline buffer (pH 7.4) and 10-µl aliquots were removed and diluted in 2.99 ml PBS. Bisbenzimidazole (16.0 µl, 20 µg/ml in distilled water) was added to each sample. After mixing and incubation at 25°C for 15 min, the fluorescence was measured in a fluorometer with excitation and emission wavelengths at 356 and 458 nm, respectively. DNA was quantified by using calf thymus DNA (1.5 µg/ml) as a standard. Data were expressed as nanomoles of fatty acid liberated per min per mg DNA (nmoles FA/min/mg DNA) and group means compared by analysis of variance (ANOVA).

Primary cultures of mammary epithelial cells

Fourth and fifth glands from mid-pregnant (day 14 post conception) CD-1 mice were excised and placed in sterile Hanks containing insulin, hydrocortisone, and prolactin, each at a concentration of 5 µg/ml, and 1% kanamycin. The tissue was minced on ice and transferred to a solution containing 0.3% collagenase (Worthington Biochemical), DNase (42 µg/ml), and the same hormones. The tissue was dissociated in a 37°C incubator with 5% CO₂ for approximately 1 h with agitation. After gentle trituration, epithelial cells were collected with three centrifugation steps and washed with subsequent removal of the supernatant at each step. Cells were plated on fetal calf serum-pretreated collagen gels (28, 29) or on basal lamina material derived from Engelbreth-Holm-Swarm mouse tumor (EHS, Matrigel™) (30) with F-12 medium containing fetuin (1.0 mg/ml), fetal calf serum (1%), penicillin-streptomycin (1.5 mg/ml), epidermal growth factor (10 ng/ml), and the hormones, prolactin, insulin, thyroxine, and hydrocortisone (all at 5 µg/ml). After 24 h the fetal calf serum was removed and bovine pituitary extract (7.0 µg/ml), transferrin (5 µg/ml), epidermal growth factor (3.3 ng/ml), and sodium selenite (87.7 ng/ml) were added along with the hormones described above. After 48 h, the collagen gel was released from the plastic culture dishes and a media containing insulin (5 µg/ml), sodium selenite (87.7 ng/ml), prolactin (3 µg/ml), and hydrocortisone (1 µg/ml) was added. The media were changed daily.

LPL activity measurements

Triplicate tissue samples (≈ 45 mg) from the mammary gland of lactating mice and from genital fat surrounding the bifurcated uterus of nonpregnant mice assigned to fed, fasted, and refed groups were quickly removed, blotted dry on shark skin filter paper, and weighed. The heparin-releasable LPL activity and DNA content were measured.

LPL immunohistochemistry

Mammary tissue pieces ($\approx 1 \text{ mm}^2$) from fully lactating mice were fixed and embedded in JB-4 resin using a modification of the method of Beckstad (31). After dissection, tissues were quickly fixed in 1% paraformaldehyde in 0.1 M Sorensen's buffer (pH 7.4) for 1 h followed by fixation in 4% paraformaldehyde for 6 h. Tissues were then transferred to a solution containing 0.1 M Sorensen's buffer with 2% sucrose and 50 mM ammonium chloride for 3 h and finally to the same solution without sucrose. Tissues were dehydrated in 75% acetone (1 h), 100% acetone (2 h), and acetone-glycol methacrylate 1:1 (1 hr). After an overnight infiltration with methacrylate (component A, JB-4 resin), tissues were embedded on ice in methacrylate with 0.45% benzolperoxide (component C) and 0.5 ml N,N-dimethylaniline in polyethyleneglycol-400 (component B).

Unless otherwise stated, all incubations were carried out overnight in a sealed humid container at 4°C. After polymerization, 2- μm thick sections were cut on an ultramicrotome and were allowed to air dry on poly-L-lysine (0.02%) coated slides. Prior to immunohistochemistry, the JB-4 plastic was permeabilized by incubating the sections in 0.25% porcine trypsin in Puck's saline A (pH 7.6) for 1 h at 37°C. After washing briefly in PBS, sections were incubated with 30 μl of 5% rabbit serum (Sigma) overnight at 4°C. The rabbit serum was removed by gentle shaking and 30 μl of primary antibody to LPL (1:100) in 2% BSA in PBS was added. This polyclonal antibody (goat anti-rat) has been shown to be specific for LPL by inhibition of LPL activity and Western blot analysis (32). This antibody was also specific for mouse LPL by Western blot (data not shown). After incubation, slides were washed in 2% BSA in PBS and rinsed in distilled water. Slides were then incubated with a rhodamine-conjugated rabbit anti-goat, F(ab')₂ fragment (Cappel) diluted 1:100 in 2% BSA in PBS. Slides were washed for 1.5 h in PBS with several buffer changes and coverslips were secured with Aqua-mount (Lerner Laboratories). Sections were photographed using an Olympus BH-2 microscope equipped with a fluorescence unit.

Riboprobe preparation

A plasmid pGEM7, containing an 868-base pair LPL cDNA, was used to generate antisense and sense riboprobes using SP6 and T7 polymerases, respectively (33). Transcription conditions were modified from Melton et al. (34) using 1 μg linearized cDNA template, 100 μCi ³⁵S-labeled UTP (Amersham) and 1 unit SP6 or T7 polymerase (Promega). This reaction was carried out for 1 h (37°C). RQ1 DNase (1 unit/ μg DNA) was then added and incubated for 15 min (37°C) after phenol-chloroform 1:1 extraction. Riboprobe was placed over a Sephadex G-50 column (Pharmacia), eluted with 100 μl of sterile Tris-

EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), counted, and precipitated overnight (-20°C).

In situ hybridization

A protocol similar to that used to detect α -casein mRNA in lactating rat mammary tissue was followed (35). Prior to tissue processing, glassware and lab utensils were baked overnight followed by autoclaving. Solutions were made using diethylpyrocarbonate-treated water followed by autoclaving. Briefly, tissues were minced and fixed for 1 h at room temperature in 0.2% glutaraldehyde and 5% paraformaldehyde in PBS, pH 7.2. After rinsing in PBS, tissues were dehydrated in graded alcohol solutions (70%, 80%, 90%, 100%). Tissues were then infiltrated with Araldite 502-propylene oxide 1:1 overnight and then immersed in Araldite 502 in Beem capsules for 6 h at room temperature. After polymerization at 56°C for 18 to 20 h, 2- μm sections were cut using an ultramicrotome. Prior to in situ hybridization, sections were deplastized in a 1:2 solution of 0.5% KOH in absolute methanol and acetone-benzene 1:1 for 45 min at room temperature. Residual KOH was neutralized in 1% acetic acid in methanol for 1 min. Slides were rinsed briefly in 2 \times SSC followed by the addition of 50 μl of hybridization buffer (50% 2 \times SSC, 50% de-ionized formamide, 1 mM EDTA, 0.2% polyvinylpyrrolidone, 0.2% ficoll, 50 mM dithiothreitol, 500 $\mu\text{g}/\text{ml}$ denatured salmon sperm, and 1 mg/ml yeast transfer RNA). After a 3- to 5-h incubation at 42°C, the hybridization buffer was removed by gentle shaking and 20 μl of hybridization buffer containing 50,000 cpm per microliter of antisense or sense riboprobes was placed on slides and incubated in a humid container at 42°C overnight. Silanized coverslips sealed with rubber cement were used to prevent evaporation. Coverslips were removed in 2 \times SSC at 50°C. Unbound probe was removed by washing in 50% formamide in 2 \times SSC, 30 min at 50°C. After rinsing twice in 2 \times SSC, slides were placed in pancreatic RNase (50 $\mu\text{g}/\text{ml}$ in 2 \times SSC) for 1 h at 37°C. Residual RNase was removed by washing twice in 2 \times SSC at 50°C for a total of 30 min. Slides were dehydrated, dipped in Ilford emulsion, and exposed for 7 days in a light-proof box at -20°C. Slides were developed (Kodak D-19) and counterstained with hematoxylin and eosin.

RESULTS

LPL activity measurements in vitro

Two culture systems were used to measure extracellular and intracellular pools of LPL. The first system was similar to that developed by Emerman and Pitelka (28). Here primary mammary epithelial cells were cultured on floating rat-tail collagen gels. These cultures formed a

monolayer of cells with basal surfaces attached to the collagen gel. When the gel was released from plastic dishes, the cells underwent the morphological change necessary for the production of milk proteins (36). In the second culture system, mammary epithelial cells were cultured on basal lamina (EHS). In contrast to the monolayer cultures, these cultures formed hollow ball structures, termed mammospheres, with basal surfaces oriented outwards and apical surfaces directed inwards toward a lumen (30). Both types of cultures synthesize and secrete milk proteins with appropriate polarity under the culture conditions described. After 5 days in culture, two experiments were performed; mammary epithelial cultures, on floating collagen gels or EHS were carefully transferred to test tubes containing F-12 medium (400 μ l) and LPL activity was measured in the medium after cells were incubated for 45 min at 37°C with various amounts of heparin (0.5, 2.0, 10.0, 20.0, 50.0 μ g/ml). In a second set of cultures, insulin at various concentrations (0.33, 0.99, 1.65, 3.33, 4.95 μ g/ml) was added for 16 h and LPL activity was measured in extracellular and intracellular compartments as described in Methods. LPL activity was detectable neither in primary epithelial cultures on floating collagen cultures nor in mammosphere cultures under the tested conditions. The amount of heparin or the amount of insulin affected neither LPL releasable by heparin nor intracellular LPL (data not shown).

Nutritional effects on the LPL activity of mammary gland and adipose tissue

Initial experiments showed that heparin-releasable LPL activity in mammary tissue from lactating mice was similar to that in adipose tissue from nonpregnant mice (Fig. 1). Ten hours of fasting decreased mammary LPL activity to the level observed in mammary tissue from nonpregnant mice (data not shown). Refeeding for 2 h increased mammary tissue LPL approximately fivefold, but levels were still fivefold less than those observed in ad libitum fed mice. Very similar effects of fasting and refeeding were observed in adipose tissue from nonpregnant mice. Genital fat in lactating animals was not present in adequate quantities for measurement of LPL activity.

Distribution of adipocytes in the lactating mammary gland

In mammary tissue from nonpregnant mice, the predominant cell type seen was mammary adipocytes with epithelial structures interdispersed among the adipocytes (Fig. 2a). At mid-pregnancy (14 days post conception), morphological changes were apparent as epithelial structures began to proliferate into the adipocytes (Fig. 2b). At full lactation (10 days postpartum), the morphology of the mammary tissue was quite different (Fig. 3C). Epithelial cells were now the predominant cell type and only small

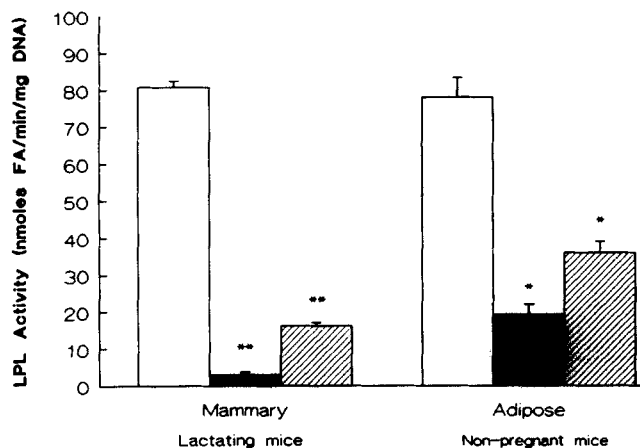


Fig. 1. Comparison of adipose and mammary tissue LPL activities released by heparin. CD-1 mice were either fed ad libitum (open bars), fasted (16 h overnight; black bars) or refed (fasted and fed 2 h; hatched bars). Heparin-releasable LPL activity (nmol FA/min/mg DNA) was measured in mammary tissue and genital adipose tissue from nonpregnant animals. LPL activity responded similarly in both tissues. Fasting decreased LPL activity in both tissues compared to fed ad libitum levels ($P < 0.01$). Refeeding increased LPL activity in adipose tissue ($*P < 0.05$) and mammary tissue ($**P < 0.01$) over levels measured in the fasting state.

channels of lipid-filled adipocytes could be distinguished because the majority of adipocytes had been depleted of their lipid stores. Milk fat globules inside lumens were also evident.

LPL immunohistochemistry

Immunohistochemistry on sectioned lactating mammary tissue revealed very distinct staining patterns for LPL. With anti-LPL serum, unequivocal fluorescence of the mammary adipocytes and light diffuse staining over epithelial cells was observed (Fig. 3A). Concentrated fluorescence was also observed as a defined band of staining in the interstitial areas between the epithelial cells (Fig. 3B). Diffuse fluorescence was associated with milk fat globules in the lumens. Immunostaining was also seen in blood vessels (data not shown). As a control, immunostaining with nonimmune goat serum revealed a low background (Fig. 3C).

LPL in situ hybridization

Hybridization with an antisense riboprobe to LPL showed dense collection of silver grains over cells located in the interstitial area between mammary epithelial cells (Fig. 4a). More scattered silver grains were localized over nuclei of some epithelial cells. The selectiveness of hybridization could be better visualized at a higher magnification where interstitial cells, located next to epithelial cells, showed clear patterns of hybridization to the LPL riboprobe (Fig. 4b). Hybridization of the sense riboprobe

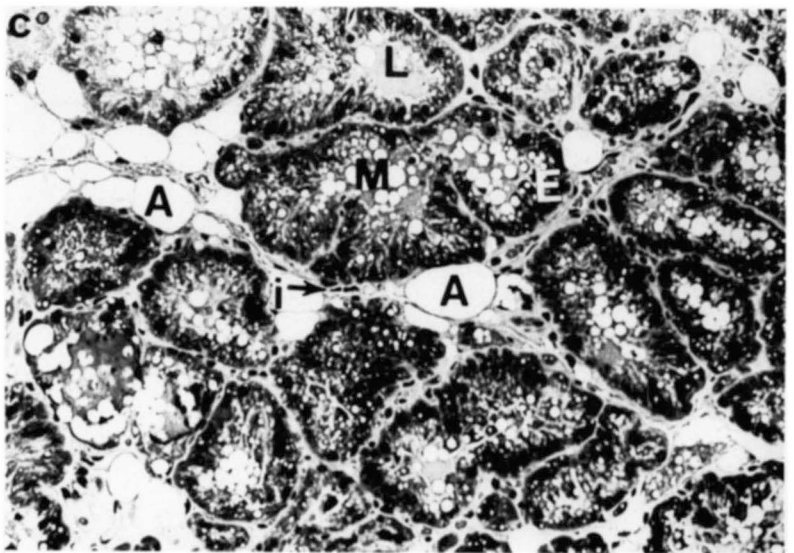
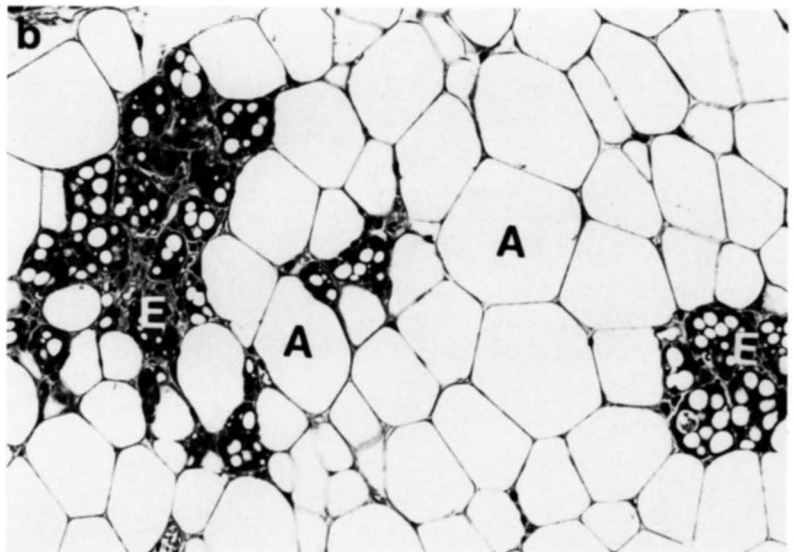
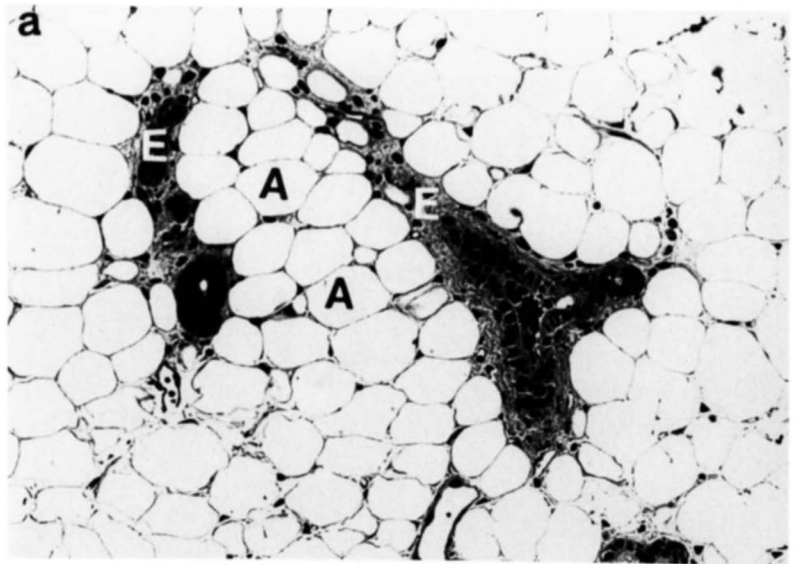


Fig. 2. Morphological changes in mammary adipocytes in preparation for lactation. Tissues were embedded in Araldite and counterstained with hematoxylin and eosin as outlined. In nonpregnant mice, adipocytes (A) were noticeable with few epithelial elements (E) present (Fig. 2a, 50 \times). At mid-pregnancy (14 days post conception), epithelial cells proliferated and grew into the mammary fat pad (Fig. 2b, 50 \times). At full lactation (10 days post partum) mammary epithelial cells lined lumens (L) containing milk (M) (Fig. 2c, 50 \times). Most of the mammary adipocytes were depleted of their lipid stores (i), however, septa with channels of lipid-filled adipocytes were evident.

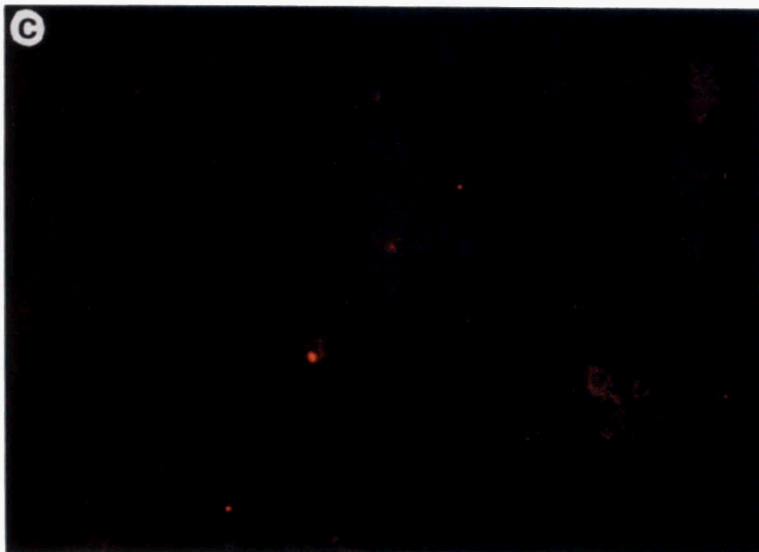
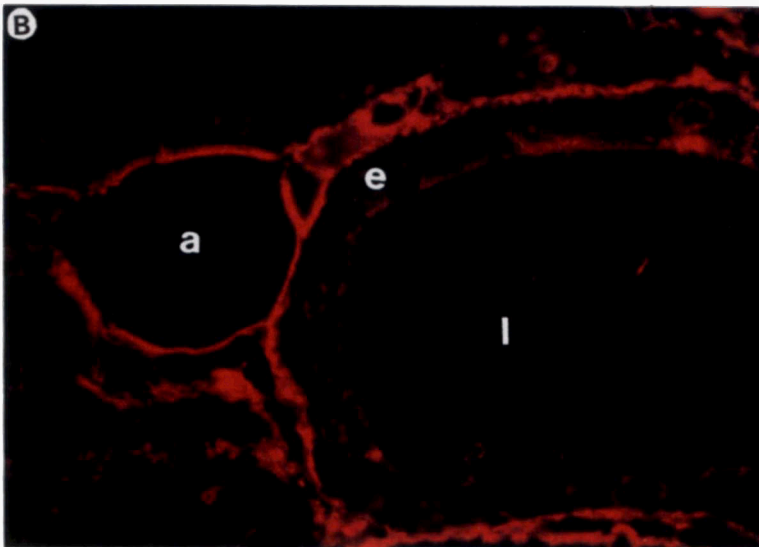
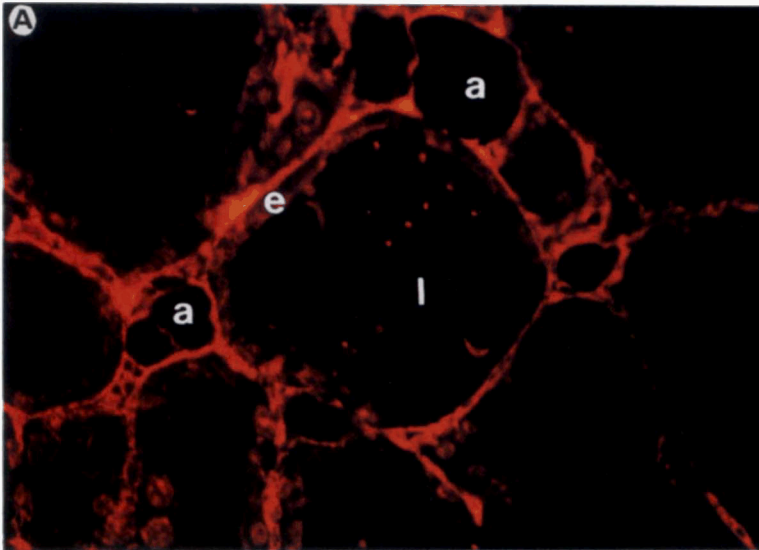


Fig. 3. Distribution of LPL in lactating mammary gland by immunohistochemistry. To maintain tissue morphology and retain milk protein, lactating mammary tissue (10 day post partum) was embedded in JB-4 plastic. Immunohistochemistry, using a polyclonal antibody to LPL, was used followed by a secondary antibody conjugated with rhodamine. Mammary adipocytes (a) showed distinct clear staining. Diffuse light staining was associated with mammary epithelial cells (e) and in milk lumens (l) (Fig. 3A, 100 \times). At higher magnification a gradient pattern of staining was evident from mammary adipocytes to epithelial cells (Fig. 3B, 250 \times). As a control, nonimmune serum was used as the primary antibody and only low background staining was observed (Fig. 3C, 100 \times). Immune and nonimmune slides were photographed at the same exposure settings.

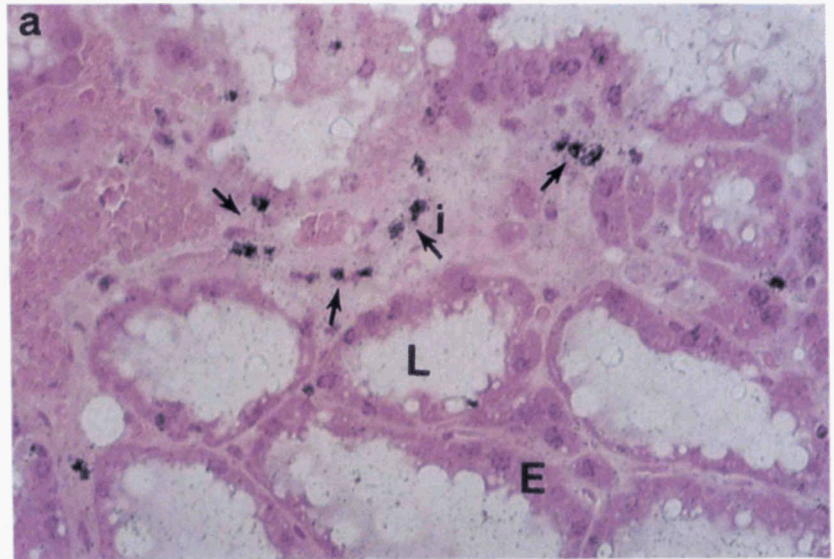
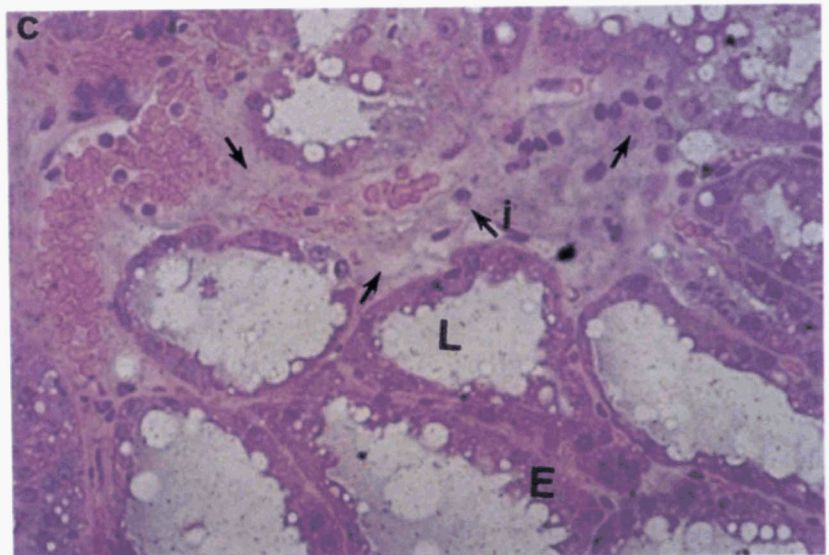
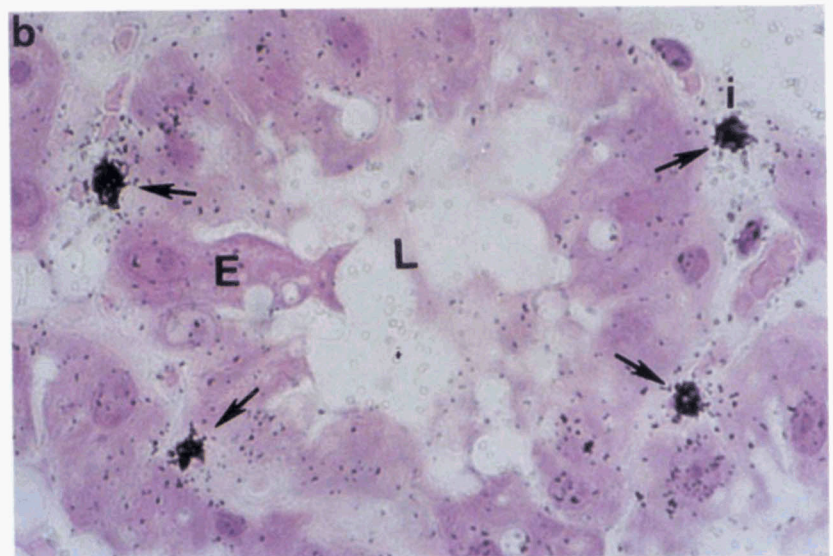


Fig. 4. Localization of the LPL mRNA in lactating mammary tissue by in situ hybridization. Ten day post partum lactating mammary tissue sections were embedded in Araldite. Concentrated areas of silver grains were seen over interstitial cells (i) in between alveolar structures (E) using an antisense [³⁵S]UTP-labeled LPL riboprobe (Fig. 4a, 100 ×). At higher magnification, four interstitial cells surrounding an aveolar structure showed discrete hybridization signal (Fig. 4b, 250 ×). Diffuse silver grains were seen, not associated with any structure, when a sense riboprobe was used as a control (Fig. 4c, 100 ×).



tended to produce a diffuse background staining which was not associated with any cellular structure (Fig. 4c).

DISCUSSION

We have obtained several lines of evidence that, taken together, strongly suggest that interstitial cells located in the connective tissue adjacent to mammary alveoli serve as a source of LPL in the lactating mammary gland. We were unable to obtain LPL in mammary cultures under conditions where adipocytes produced the enzyme. Highly concentrated LPL immunostaining was observed in the mammary stroma and LPL mRNA appeared to be localized in mammary interstitial cells.

Our inability to observe LPL activity in cultured mammary epithelial cells could be due to a deficiency in the media that prevents production or secretion of LPL *in vitro* even though these cells make milk proteins (30, 36). However, 3T3-L1 fibroblasts (37), Ob17 cells (mouse preadipocytes (38)), rat adipose tissue-derived stromal cells (39), and cultured human adipocytes (40) produce LPL *in vitro*. Secondly, in other tissues, the secreted form of LPL is transported and bound to adjacent endothelium (16). Accordingly, if LPL were produced by epithelial cells on floating collagen cells and secreted through the basal surface, the use of a collagen gel may interfere with this process. This seems unlikely because mammosphere cultures, with basal surfaces oriented outwardly, also failed to produce active LPL. A third possibility is that mammary epithelial cells, which make up greater than 95% of the cells in our cultures (data not shown), may not be responsible for the production of LPL *in vivo*.

In earlier experiments (Jensen, D., and M. C. Neville, unpublished results) we took care to optimize the fixation and embedding procedures used for the lactating mammary gland so that the highly soluble milk proteins would be retained within the tissue and lumens. It seems likely that these conditions should maintain the physiologic distribution of LPL. Further, the technique used maintains excellent tissue morphology and allows good access of antibody to the antigen. We observed a strong gradient of fluorescence within the tissue section with intense fluorescence over interstitial areas including mammary adipocytes. More diffuse and lighter staining of mammary epithelial cells and milk lumens was observed. This staining pattern suggests that LPL is first transported from its site of synthesis into the interstitial spaces and to capillary lumens where it is functional. Interstitial LPL may then be taken up by the mammary cells and transported to the milk by transcytosis. The appearance of LPL in milk has been well documented in many species, but its function there remains unclear (41). Although the immunohistochemical data cannot pinpoint the cell of origin of LPL, they are consistent with an interstitial ori-

gin of the enzyme. We are at a loss to explain the differences between our results and the results of immunohistochemical localization of LPL in mammary gland of the lactating guinea pig recently published by Camps et al. (22). In contrast to our results, the authors showed strong fluorescence only over the apical poles of the epithelial cells and in the capillary endothelium. Camps et al. (22) did not give any evidence that their animals were indeed in full lactation, a potential problem in guinea pigs where the young suckle for 7 days or less.

In our hands *in situ* hybridization produced a strong signal over cells in interstitial areas immediately adjacent to mammary alveoli in the lactating mouse mammary gland. There was no increased signal over cytoplasmic areas of alveolar cells nor in connective tissue septa, leading us to suggest that these interstitial cells are a primary site of origin of mammary LPL. The nature of these cells is not clear.

A mammary fat pad is necessary in order for lactation to be established (42). In pubertal mice, mammary epithelium grow into a specialized fat pad where the ducts are evenly interspersed in a bed of adipose tissue. With pregnancy the epithelial structures grow into alveoli that still maintain an intimate association with adipose tissue. These adipocytes regress at lactation, losing the majority of their fat, but maintain a position interspersed among the now very extensive alveolar complexes. Only small channels of lipid-filled adipocytes could be distinguished because the majority of adipocytes had been depleted of their lipid stores. The function of this fat pad has not been well understood. One possibility is that these fat-depleted adipocytes are the interstitial cells we observed that showed hybridization with the LPL riboprobe and are a source of the LPL needed for milk fat synthesis. We believe it most likely that they represent the fat-depleted adipocytes identified much earlier by Elias, Pitelka, and Armstrong (43) in interstitial areas of lactating mouse mammary glands. On electron microscopy these cells contain small fat droplets and their plasma membranes are lined with vesicles, some of which appear to open to the exterior of cells as if in the process of exocytosis. It is tempting to speculate that these vesicles are involved in LPL secretion, but there is no firm evidence to support this.

The recent evidence provided by Camps et al. (22) suggesting that epithelial cells may be the source of LPL in guinea pig lactating mammary gland needs further evaluation. In their study, silver grains were generally localized over alveolar areas. However, at the magnification presented, discrete morphological areas were difficult to distinguish. On closer inspection some silver grain foci appeared to be localized between alveoli as we have seen. Other foci appeared over milk lumens, where mRNA would not be expected. In our study, the tissue-embedding protocol utilized in the *in situ* hybridization

maintained excellent tissue morphology especially in interstitial areas. This may account for the differences between the study of Camps et al. (22) and ours.

In conclusion, similar responses of LPL to nutritional stresses in mammary tissue from lactating mice and adipose tissue from nonpregnant mice, lack of active LPL in mammary epithelial cultures, intense immunohistochemical localization associated with mammary adipocytes, and strong hybridization signal over interstitial cells strongly suggest that another cell type located in the interstitial areas of the mammary gland is responsible, at least in part, for the production of LPL in mammary tissue of mice. If the site of origin of LPL is the mammary adipocyte, as we have speculated, other questions remain unanswered such as why the mammary adipocyte loses its lipid even though it makes significant amounts of LPL. Furthermore, is this lipid depletion of the adipocyte a stimulus for LPL production or are there mammary-specific factors responsible for the reciprocal regulation of LPL in mammary adipose tissues during lactation? Experiments such as co-culturing of adipocytes and mammary epithelial cells may be necessary to conclusively determine the nature of these cells and to understand the regulation of LPL synthesis during lactation. ■

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