# Lipoprotein lipase mRNA in neonatal and adult mouse tissues: comparison of normal and combined lipase deficiency (cld) mice assessed by in situ hybridization

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**Abstract** Combined lipase deficiency (cld) is a genetic abnormality in mice resulting in the production of enzymatically inactive lipoprotein lipase (LPL). After suckling, these mice have markedly elevated levels of circulating triglyceride. An alteration of LPL gene expression in cld mice may affect the amount and/or the distribution of LPL mRNA in different cell types. Therefore, we performed in situ hybridization for LPL mRNA in tissues from normal and cld pups and adult mice using an antisense $^{35}$S-labeled cRNA probe. LPL mRNA had the same pattern of distribution in both cld and normal newborn mice; the probe hybridized strongly to pyramidal neurons of the hippocampus, heart myocytes, and hepatocytes. Despite the lack of noticeable fat stores, LPL mRNA was found in the dermal layer of the skin of cld mice and normal littermates. In adult mice, the cRNA probe for LPL hybridized to the hippocampus, to the heart, and to localized areas of the kidney. We conclude that despite great variation in plasma triglyceride levels, LPL gene is similarly expressed in animals with or without LPL activity. —**Yacoub, L. K., T. M. Vanni, and I. J. Goldberg.** Lipoprotein lipase mRNA in neonatal and adult mouse tissues: comparison of normal and combined lipase deficiency (cld) mice assessed by in situ hybridization. *J. Lipid Res.* 1990. **31:** 1845–1852.

**Supplementary key words** brain • hippocampus • heart • kidney • triglyceride • liver • hepatic triglyceride lipase

Lipoprotein lipase (LPL) is synthesized mainly in muscle and fat cells and hydrolyzes lipoprotein triglyceride primarily while bound to the endothelial cell lumen (1). Recently, LPL cDNA clones for mouse (2), human (3), and guinea pig (4) have become available, allowing correlation of LPL activity with LPL gene expression. LPL activity is found in several tissues other than adipose and muscle (5); LPL mRNA is also present in many organs (6, 7). Studies from our laboratory (8) demonstrated LPL mRNA in localized regions of several tissues, including brain and kidney, not thought to be important for hydrolysis of triglycerides in circulating lipoproteins. The physiological role, if any, of brain and kidney LPL is unknown. LPL in these areas may perform functions other than hydrolyzing lipoprotein triglyceride.

Regulation of LPL activity with physiologic and pharmacologic maneuvers is an area of active investigation. LPL activity in fat and muscle is differentially regulated; feeding increases LPL activity in fat and decreases LPL activity in muscle (1). In fed animals, increased fat LPL activity may cause greater uptake of circulating triglycerides for storage by fat cells; during fasting, decreased adipose LPL may permit more triglyceride to be taken up by muscle cells where it is used for oxidative metabolism. Three observations suggest a post-translational regulation of LPL activity in fat tissue. *1*) Synthesis of LPL protein is the same in adipocytes isolated from fed or fasted animals (9). *2*) LPL activity increases with feeding, but no change in LPL protein or LPL mRNA was found in human adipose biopsies (10). *3*) In rats, LPL activity decreases during fasting but LPL protein and mRNA paradoxically increase (11). In contrast, insulin increases LPL activity and mRNA levels in isolated fat cells (12, 13).

Plasma insulin levels increase in the postprandial period; however, the effects of insulin and feeding on LPL activity and mRNA differ. An additional plasma factor, perhaps triglycerides or fatty acids, may therefore be involved in the response to feeding and may counter the stimulating effects of insulin on adipose LPL gene expression (14).

The combined lipase deficiency (cld) mouse is an animal in which both LPL and hepatic triglyceride lipase

Abbreviations: LPL, lipoprotein lipase; cld, combined lipase deficiency; HTGL, hepatic triglyceride lipase; RBP, retinol-binding protein; PBS, phosphate-buffered saline.

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(HTGL) activities are nearly absent. Cld mice develop severe hyperchylomicronemia with suckling and die within 2–3 days postpartum. A single recessive mutation on chromosome 17 results in the production of inactive LPL and hepatic triglyceride lipase (15). Intracellular levels of LPL protein are, however, increased in tissues from these mice (16). Davis, Doolittle, and Ben-Zeev (17) and Masuno et al. (18) have demonstrated that processing of the lipase protein in cld mice is impaired with retention of HTGL and LPL in the endoplasmic reticulum. Cld mice develop high plasma levels of triglyceride but are unable to generate free fatty acids in the circulation. Thus, the importance of plasma triglycerides and free fatty acids on LPL gene expression in the postnatal period could be assessed. Using in situ hybridization for LPL mRNA, we compared the distribution of LPL mRNA in neonatal and adult mice, and cld pups. Control studies were performed using sense strand LPL cRNA and cRNA for retinol-binding protein (RBP).

## METHODS

### Animals

Mice carrying the cld mutation were obtained from Dr. Karen Artzt, Laboratory of Developmental Genetics, University of Texas, Austin. This strain of mice inherited a defect in tail embryogenesis (tailless); however, they were back-crossed so the cld/cld defect could be detected at birth by the presence of a tail. The mice were bred in the animal care facility at Columbia University. Newborn mice were allowed to suckle; then they were killed within 6–12 h after birth. Cyanotic and hypoactive mice with a tail were identified as cld/cld and this designation was verified by the presence of lipemic blood in the pups that were killed. Tailless, normoactive, and pinkish mice from the same litter (normal littermates) and adult mice were also used for these studies.

### Preparation of tissues

Cld pups and normal littermates were processed in a similar fashion. The mice were decapitated and blood was collected from the neck into heparinized capillary tube. Plasma cholesterol and triglyceride measurements were performed using enzymatic methods on an automated analyzer (ABA 100, Abbott, Chicago, IL). The heads and bodies were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) (2.7 mM KCl, 1.5 mM $KH_2PO_4$, 137 mM NaCl, 8.1 mM $Na_2HPO_4$, pH 7.4). The next day the tissues were placed in 15% sucrose in PBS until they submerged. The two parts were embedded in OCT compound (Miles Laboratory, Naperville, IL) and slowly frozen in isopentane cooled in liquid nitrogen. The tissues were then stored at −70°C and sectioned within 1 week after freezing.

Tissues from adult mice were fixed in situ. The mice were anesthetized by $CO_2$ inhalation, the heart was exposed, and a catheter was introduced into the left ventricle. A second catheter, used for outflow, was placed into the right atrium. The animals were perfused with 15 ml of cold (4°C) PBS, followed by 15 ml of 4% paraformaldehyde in PBS. The organs were then isolated, fixed in 4% paraformaldehyde overnight, and further processed as described above for neonatal animals.

### Preparation of sections

Glass slides were coated twice with poly-L-lysine and 5- to 10-μm sections were obtained using a cryostat (Bright 5030 microtome) at −20°C. The slides were then stored at −70°C in air-tight boxes containing desiccant capsules. Axial sections of the neonatal mouse heads, cross sections of the neonatal bodies, coronal sections of the adult brains, and cross sections of adult heart, liver, and kidney were obtained.

### Labeling of LPL and RBP cRNA

LPL cRNA probe preparation was described previously (8). In brief, a 624 base pair fragment was isolated by Bam H1 from LPL mouse cDNA clone ML5 (2) and subcloned into the Bam H1 site of PGEM 4 (Promega Biotec, Madison, WI). The cDNA fragment used for preparation of the cRNA probe for rat RBP has been described by Soprano et al. (19). One hundred μCi of $^{35}$S-labeled UTP (sp act 1000–1400 Ci/mmol, New England Nuclear, Boston, MA) was used to label the RNA probes transcribed from 1 μg of linearized cDNA. Transcription was performed in both the sense and the anti-sense directions by the use of SP6 and T7 RNA polymerase (Boehringer Mannheim, Indianapolis, IN). The probe was extracted with phenol and precipitated twice with ethanol; an aliquot was then run on a 6% polyacrylamide gel to check the integrity of the cRNA. The labeled probes were stored at −70°C and used within 3 weeks.

### In situ hybridization

In situ hybridization was performed as described in detail previously (8). Briefly, the sections were thawed and immediately treated with proteinase K (1 μg/ml) in 20 mM Tris-HCl (pH 7.5), 2 mM $CaCl_2$, for 10 min at room temperature. The slides were then washed with several solutions: *1*) five min with 0.1 M triethanoleamine, pH 8.0; *2*) 10 min with 0.25% acetic anhydride in 0.1 M triethanoleamine, pH 8.0; and *3*) 10 min with 2 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). Sections were then prehybridized at 55°C for 2 h with a buffer containing 0.5 mg/ml salmon sperm DNA, 50% formamide, 0.6 M NaCl, 0.01 M Tris (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.1% bovine serum albumin, 1 mM ethylenediaminetetraace-

tate, 0.5 mg/ml total yeast RNA, and 50 μg/ml yeast transfer RNA. Hybridization was performed overnight at 55°C using the prehybridizing buffer in addition to 10 mM dithiothreitol, 10% dextran sulfate, and 2500 cpm/μl $^{35}$S-labeled UTP RNA probe. Slides were then washed for 30 min with 2 × SSC at room temperature and single stranded RNA was digested at 37°C for 45 min with RNAase A (20 μg/ml) and RNAase T1 (2.2 μg/ml) (Sigma Chemical Co., St Louis, MO). Finally, the slides were washed with 0.1 × SSC at 55°C, dehydrated in graded alcohol, vacuum-dried, and exposed to photographic film for 2 days at room temperature. After exposing the film to detect positive hybridization, the slides were dipped in emulsion (NTB-2 Nuclear track emulsion, Eastman Kodak, Rochester, NY), placed for 4 weeks at 4°C in light-resistant slide boxes containing desiccant, developed, fixed, and stained with hematoxylin and eosin. Slides processed with the sense [$^{35}$S]RNA probe were used as a control.

### Photography

The slides were viewed and photographed at 120–1200 × magnification. Using dark field illumination, the exposed emulsion appeared as white silver grains. Under bright field microscopy, performed to allow better identification of the cells, dark grains would indicate areas of positive hybridization.

## RESULTS

### Plasma lipid levels

Blood drawn from cld mice was severely lipemic and readily distinguishable from blood obtained from normal littermates. For this study, we observed a total of six litters with 37 pups, an average of 6 per litter. Of the total 37 mice, 28 were normal and 9 were cld/cld homozygotes. The average plasma lipid levels for the cld mice were (n = 5) 296 ± 158 mg/dl for cholesterol and 3601 ± 480 mg/dl for triglyceride (mean ± standard deviation). Normal littermates (n = 5) had cholesterol levels of 63 ± 7 mg/dl and triglycerides levels of 81 ± 8 mg/dl. These values correspond to the levels reported by Paterniti et al. (15), who also noted that triglyceride levels of cld mice increased to >20,000 mg/dl at 48 h after birth.

### Brain LPL mRNA distribution

**Fig. 1** shows axial sections of neonatal normal and cld brains. These sections were obtained by cryosectioning

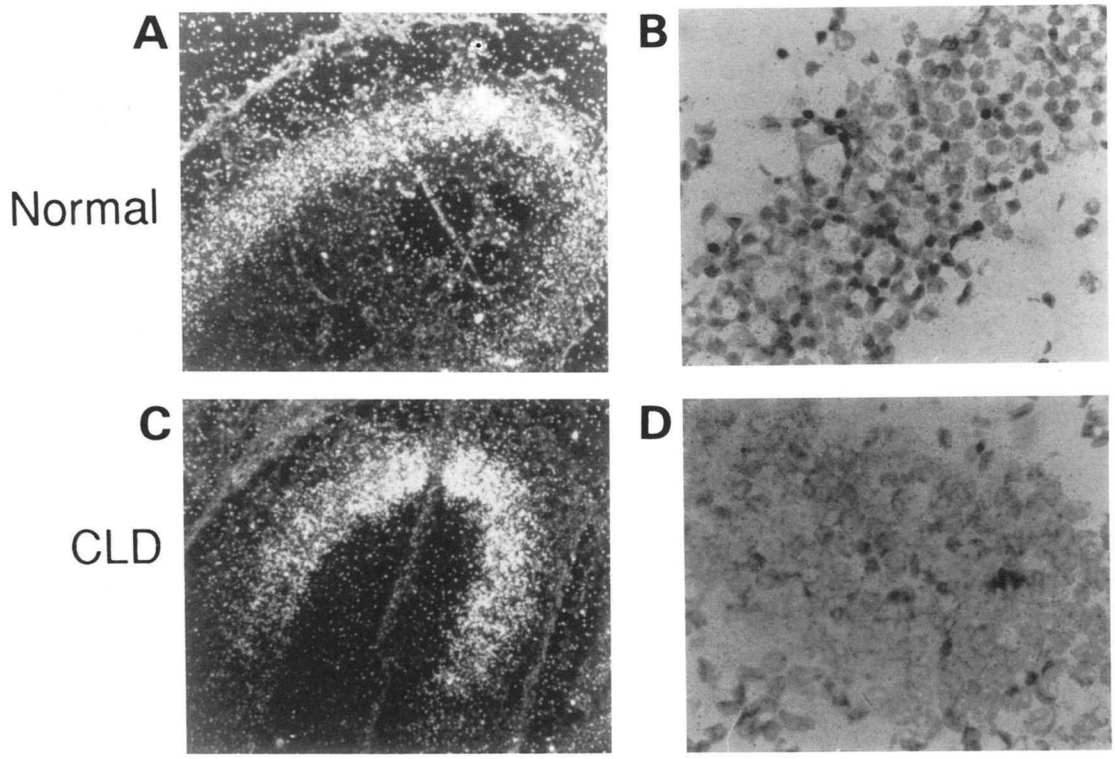


**Fig. 1.** In situ hybridization for LPL mRNA in neonatal mice. Shown are dark field and bright field illumination photographs of brains from normal littermates (A and B) and cld mice (C and D). The dark field photographs (A and C) were taken at 300 × magnification. Bright field photographs (B and D) were taken at 1200 × magnification. The hippocampus, the crescent-like structure shown in A and C, appears silver due to polarized light epiluminescence indicating hybridization with the $^{35}$S-labeled LPL cRNA probe. In the bright field photographs (B and D), positive hybridization is indicated by the dark dots overlying the pyramidal neurons identified here by their characteristic clear areas.

the entire head of the animals. Hybridization of the LPL cRNA produced a positive signal seen as white areas in the dark field photomicrographs (A and C). An intense signal, indicating LPL mRNA, was found in the pyramidal neurons of the hippocampus. Similar findings were reported by our group in studies of 5-day-old rat brain (8). Using tissues from cld or normal mice, the amount and location of hybridization appeared similar.

The role of LPL enzyme in the brain is unknown. LPL in the postnatal period may be important for lipid accumulation in the developing brain. In this regard, brain morphology of cld mice has not been reported. When viewed under bright field illumination (Fig. 1, B and D), there were no obvious structural differences between normal and cld brains.

### Heart LPL mRNA

In situ hybridization for LPL mRNA was performed on cross sections of normal and cld bodies and sections were chosen which contained heart tissue (**Fig. 2**). The LPL cRNA probe hybridized strongly to the heart, indicating the presence of LPL mRNA (Fig. 2, A and D). This area of intense hybridization was readily apparent on the X-rays taken prior to dipping the slides in photographic emulsion. Photographs from control studies using sense cRNA probe are shown in Fig. 2, B and E. High power magnification using bright field illumination is shown in Fig. 2, C and F. The dark dots, indicating LPL mRNA, are within myocytes of normal and mutant mice.

### Liver LPL mRNA

LPL activity and protein are found in neonatal rat liver (5, 20) and LPL activity was described in liver of young roosters (21). We used in situ hybridization to demonstrate LPL mRNA in neonatal mouse liver. **Fig. 3A** shows dark field illumination of a section from a cld mouse liver; a control slide hybridized with the sense RNA probe is shown in Fig. 3B. Fig. 3C shows the same section using bright field illumination. This high power, bright field photograph shows dark dots, indicating LPL mRNA, within the cld hepatocytes. Slides hybridized with the RBP probe as a positive control, are shown in Fig. 3, D, E, and F. Using similar amounts of probe and identical hybridization conditions, hybridization for RBP mRNA appeared greater than that for LPL mRNA.

### LPL mRNA in skin

The skin and subcutaneous tissues from normal and cld mice were studied. Dark field photographs are shown in **Fig. 4, A and C**. Hybridization, indicating LPL mRNA, is obvious in the dermal layer of the skin of both mice. Under bright field illumination (Fig. 4, B and D) the epi-

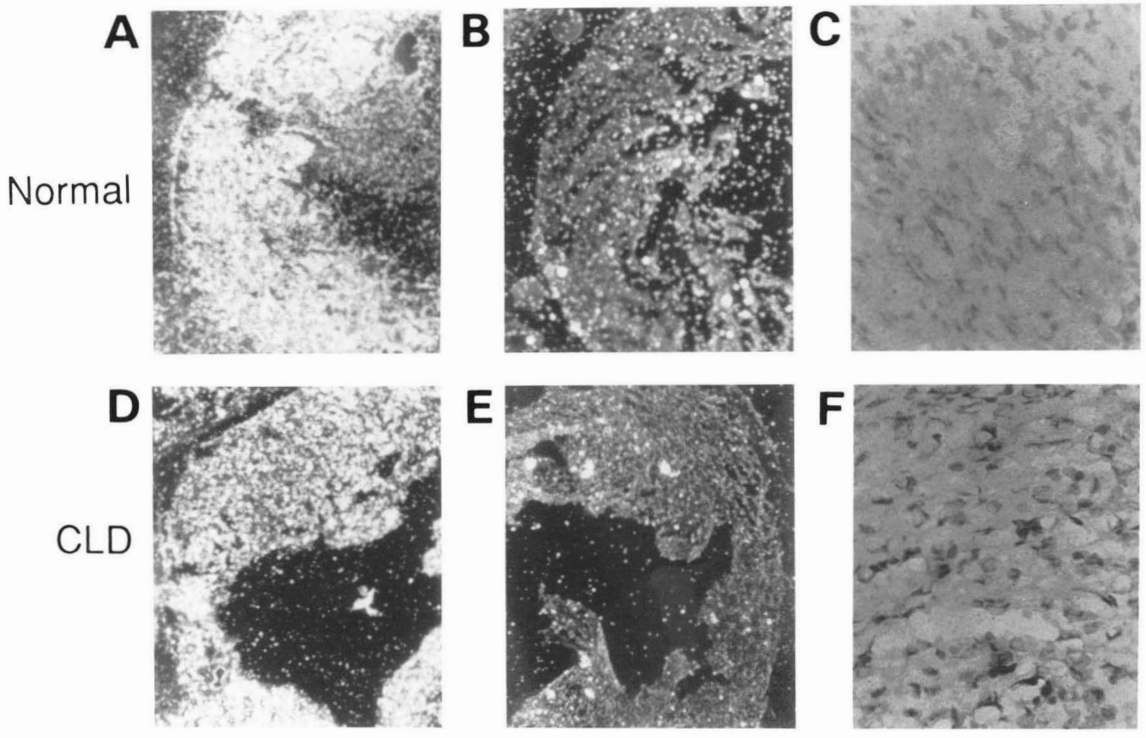


**Fig. 2.** Localization of LPL mRNA in normal (A, B, C) and cld (D, E, F) neonatal hearts. The $^{35}$S-labeled LPL cRNA probe hybridizes strongly to the heart in both animals as evidenced by the dark field photographs, A and D, taken at 300 × magnification. The silver grains are present in the ventricular wall; the dark area in the center represents the cardiac lumen. Dark field photomicrographs, B and E, show in situ hybridization studies using sense LPL $^{35}$S-labeled cRNA probes and confirm the specificity of the signal. In photographs C and F, taken under bright field illumination at 1200 × magnification, the dark dots representing LPL mRNA are located within the myocytes of both the normal and the cld hearts.

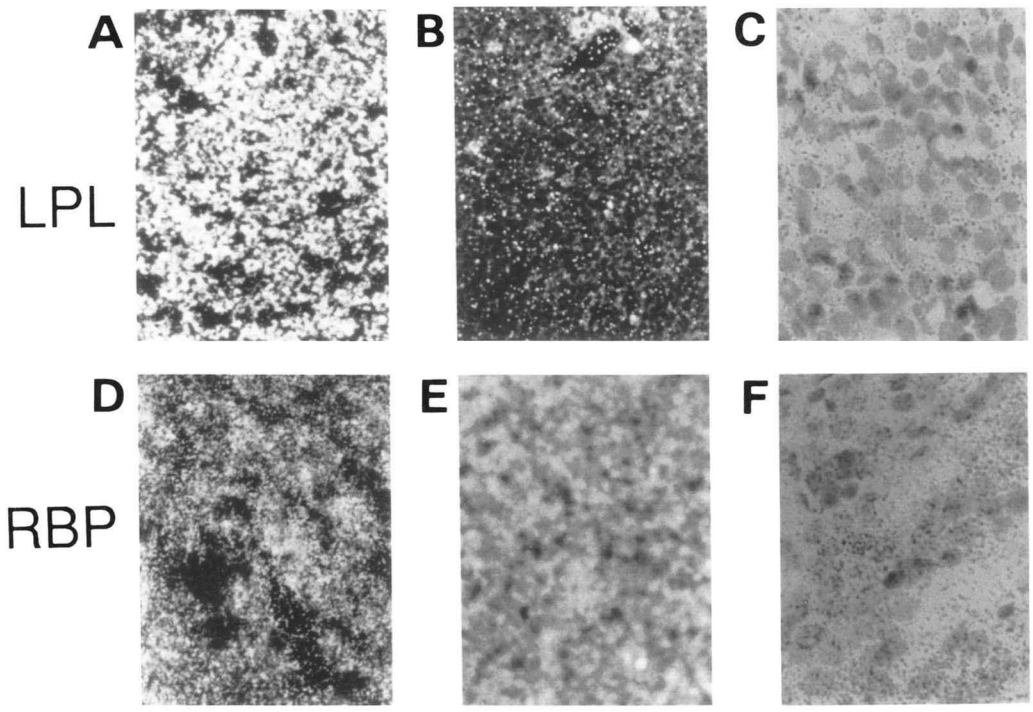


**Fig. 3.** In situ hybridization for LPL mRNA and RBP mRNA in neonatal livers. In the dark field photomicrograph, A, taken at 300 × magnification, the silver grains indicating LPL mRNA are overlying the entire section of the cld liver. RBP mRNA is also evident over the normal neonatal liver in photograph D (300 × magnification). Control studies using sense strand RNA are shown in photographs B and E. In the bright field photomicrographs (C and F, 1200 × ), stained with hematoxylin and eosin, dark dots are seen overlying hepatocytes.

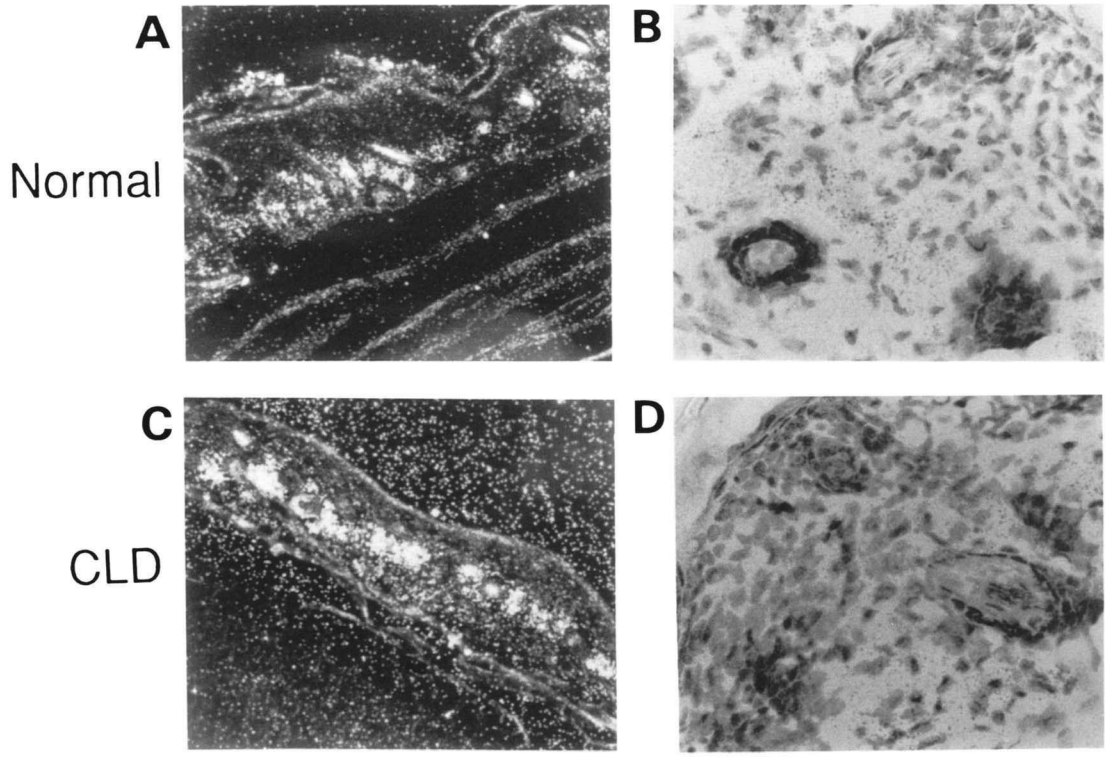


**Fig. 4.** In situ hybridization of normal and cld mouse skin with $^{35}$S-labeled cRNA LPL probe. In photographs A and C, the white dots representing LPL mRNA are seen in the dermis of the normal (A) and cld (C) skin. Bright field photographs are shown in B and D. In these sections stained with hematoxylin and eosin, LPL mRNA is apparent as dark dots in the dermal layer.

dermis of both normal and mutant mice is thin, two to three cell layers thick, with a dermis containing well-developed hair follicles. The dark grains, representing LPL mRNA, do not seem to localize within a specific dermal cell type. The area of hybridization contains predominantly fibroblasts. The hypodermis or subcutaneous tissue in these neonatal animals is underdeveloped with no obvious adipocytes or fat stores. It is possible, however, that small amounts of fat were removed during the alcohol washes used to process these slides.

### Adult mice

Several tissues from adult mice were hybridized with the LPL cRNA probe to compare LPL distribution in adult mouse tissues with that in the neonatal mouse. In addition, hybridization for LPL in mouse tissues was compared to that previously published for rat tissues (8). As described in neonatal mice (Fig. 1) and 5-day-old rat brain, LPL mRNA was localized to the hippocampus (**Fig. 5A**). Hybridization of mouse kidney with the cRNA probe for LPL produced a much stronger signal than we had observed for rat. A strong signal for LPL is apparent in the outer zone of the medulla (Fig. 5B). However, we could not be certain whether hybridization also occurred to a lesser extent in other areas of this kidney. In contrast to the neonatal liver, no hybridization for LPL mRNA was found in the adult mouse liver (data not shown). As expected, hybridization, indicating the presence of LPL mRNA, occurred with heart tissues (Fig. 5C). Under bright field microscopy, dark dots, indicating silver grains, were found overlying myocytes (Fig. 5D).

## DISCUSSION

In this report, we describe the distribution of LPL mRNA in tissues from normal neonatal, cld, and adult mice. Our data, although qualitative, does not show major differences in LPL mRNA levels in tissues from normal and cld pups. Thus, our results are similar to that of Davis et al. (17), who found no difference in LPL mRNA levels, compared to actin, in several tissues from normal and cld pups. Together, these data imply that hypertriglyceridemia due to defective lipolysis does not grossly affect LPL gene expression (see below). Such a conclusion, however, should be taken with caution because the genetic defect responsible for alterations in the processing of LPL and HTGL conceivably might affect a number of other cellular proteins, some of which could be involved in LPL regulation. Aside from the comparisons between normal and cld mice, new information on LPL mRNA distribution in several tissues from neonatal and adult mice is provided in this report. This data can be compared to that for LPL mRNA distribution in rat tissues (8) and during development (7).

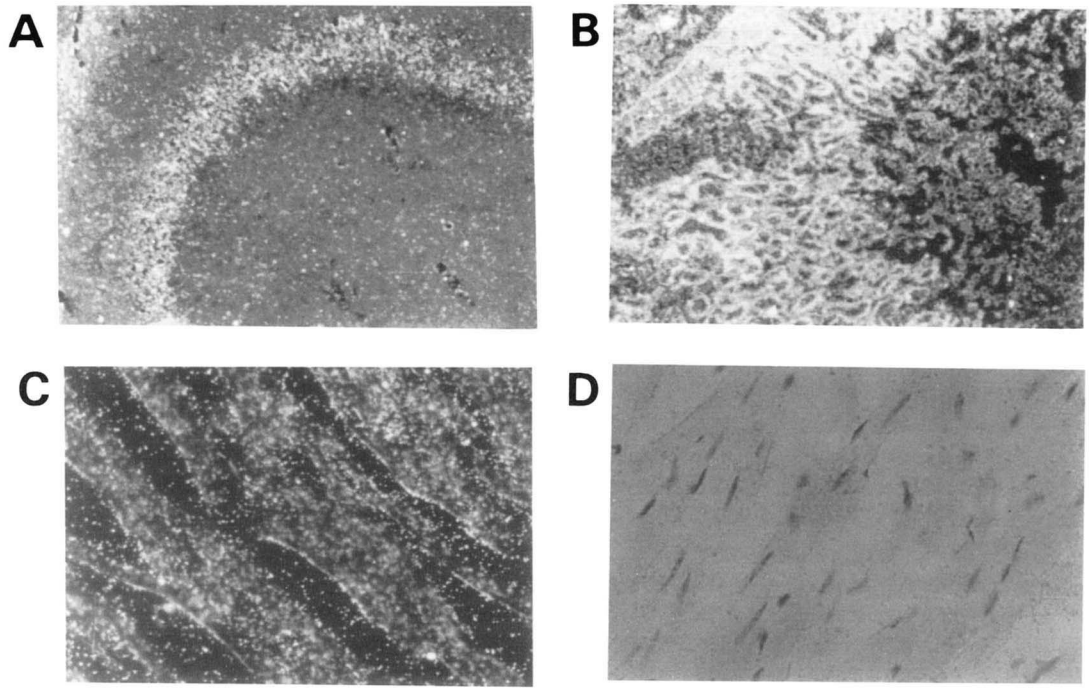


**Fig. 5.** LPL mRNA by in situ hybridization of selected adult mouse tissues. Shown are dark field photographs from brain with silver grains localizing to the hippocampus (A), kidney with intense hybridization in the outer zone of the medulla (B), and heart with silver grains localizing to the myocytes (C). The bright field photomicrograph shown in D, demonstrates hybridization for LPL mRNA in adult mouse myocytes. Control studies using sense $^{35}$S-labeled cRNA were performed with each tissue to confirm the specificity of the signal (data not shown).

LPL mRNA was found in the hippocampus of neonatal and adult mice as well as in neonatal mice deficient in LPL activity. Using immunohistochemistry, Vilaro et al. (22) recently demonstrated increased staining for LPL immunoreactive protein in the hippocampus and several other areas of the brain. The role of LPL in brain function is unclear. The hippocampus is part of the limbic system which is involved in the control of emotion, attention, drive, and memory. Chajek, Stein, and Stein (5) reported that LPL activity in rat brain increases between birth and 10 days of age and suggested that fatty acids derived from LPL-mediated hydrolysis of triglyceride might be required for increased membrane phospholipid biosynthesis during the perinatal period. By light microscopy, there were no differences in brain morphology between the normal mouse and the mouse deficient in LPL activity. This suggests that LPL activity is not critical in ensuring normal brain development during the neonatal period. Unlike humans with LPL deficiency, triglyceride hydrolysis by HTGL is also defective in these mice. Therefore, fatty acid generation by either of these two enzymes does not appear to be important for brain development in utero.

LPL activity in rat hearts increases postnatally, from birth until 20 days of age (5). This increase coincides with the development of myocytes and increased numbers of capillaries. Semenkovich et al. (7) showed that LPL mRNA increases postnatally, suggesting that increased LPL in heart tissue after birth is due, at least in part, to increased LPL gene expression. Our data, obtained using in situ hybridization, further demonstrates that LPL mRNA is found in heart myocytes in the neonatal normal and cld mice, as well as in adult animals. Whether LPL mRNA induction in myocytes involves circulating triglycerides, fatty acids, or other metabolic or hormonal factors is unclear. In this regard, Davis et al. (17) reported that LPL mRNA levels in hearts of cld mice were comparable to levels in unaffected mice. Thus, elevated levels of circulating triglycerides alone do not appear to regulate LPL gene expression.

Although LPL activity is found in the adult liver, much of this LPL may be synthesized in extrahepatic tissues. Circulating LPL associated with lipoproteins is taken up and eventually catabolized by the liver (23). Some of this LPL is catalytically active and likely accounts for much of the liver LPL activity. In fetal and newborn rodents, however, LPL is synthesized within the liver and LPL protein was detected by immunohistochemistry on hepatic endothelium (20). The cellular site of LPL synthesis, however, could not be identified. Burgaya et al. (24) demonstrated that LPL activity in neonatal liver is present in the hepatocytes and concluded that active LPL is synthesized within neonatal hepatocytes and not within hematopoietic cells. Our findings confirm the presence of LPL mRNA within hepatocytes. The diffuse signal for LPL mRNA, however, made it difficult for us to determine whether any LPL mRNA was also present in Kupffer cells. No hybridization for LPL mRNA was found in the adult liver, confirming that a developmental loss of hepatic LPL gene expression occurs. In addition, this finding verifies that the LPL cRNA probe did not cross-hybridize with HTGL mRNA.

The physiologic role of LPL in neonatal liver is still unclear. Llobera, Montes, and Herrera (25) suggested that neonatal liver LPL removes circulating triglycerides, allowing for temporary storage of fat. In the adult animal, fat storage is performed by extrahepatic tissues. Under some circumstances, for example during inflammation which is mimicked by infusion of tumor necrosis factor into animals, LPL gene expression in the liver may recur (26). Thus, the distribution of LPL may channel circulating fat calories away from peripheral storage when liver synthetic functions are increased.

LPL gene expression in subcutaneous tissue from neonatal normal and cld mice was similar; both the location and amount of hybridization were indistinguishable using tissues from each type of animal. The signal for LPL mRNA was located within the dermis. We could not conclusively differentiate fibroblasts from mast cells or monocyte-derived cells, all present in the dermis. Some dermal fibroblasts migrate to the hypodermis or subcutaneous tissue and those cells containing LPL mRNA may be pre-adipocytes. Using dot blot analysis, Oka et al. (27) reported that the ratio of LPL mRNA to total RNA was increased in brown adipose tissue from cld pups. These results may, in part, be due to the tissues analyzed and the methods used for mRNA quantification. Fat is composed of multiple cell types and the relative amounts of mRNA alone may not reflect LPL expression in a single cell type. In this situation, in situ hybridization for mRNA provides additional information about cellular events which cannot be assessed by mRNA levels.

In our previous study, we showed that LPL mRNA is present in the outer zone of the adult rat kidney medulla. Kirchgessner et al. (6), using dot blot analysis, detected greater amounts of LPL mRNA in adult mouse kidney than in rat kidney. Their findings correspond to ours. LPL mRNA was detected over the entire kidney but the signal was more intense over the outer zone of the medulla. Paradoxically, fatty acid oxidation is the principal energy yielding process in the kidney cortex (28), whereas most LPL activity is in rat kidney medulla (29). The role of kidney LPL is not clear. Whether the function of the enzyme is to hydrolyze triglycerides in circulating lipoproteins, providing fatty acid for local energy use, is uncertain.

In summary, LPL mRNA was found in a specific area of neonatal and adult mouse brain, in neonatal hepatocytes, and in mouse heart and kidney. The physiologic

role of LPL in several of these tissues requires further study. In addition, LPL mRNA distribution was similar in normal and in cld mice, suggesting that LPL mRNA expression is not affected by circulating triglyceride levels or the actions of LPL and HTGL to hydrolyze triglyceride in circulating lipoproteins.

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