Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation, and evolution

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Abstract Lipoprotein lipase (LPL) and hepatic lipase (HL) enzyme activities were previously reported to be regulated during development, but the underlying molecular events are unknown. In addition, little is known about LPL evolution. We cloned and sequenced a complete mouse LPL cDNA. Comparison of sequences from mouse, human, bovine, and guinea pig cDNAs indicated that the rates of evolution of mouse, human, and bovine LPL are quite low, but guinea pig LPL has evolved several times faster than the others. ^{32}P-Labeled mouse LPL and rat HL cDNAs were used to study lipase mRNA tissue distribution and developmental regulation in the rat. Northern gel analysis revealed the presence of a single 1.87 kb HL mRNA species in liver, but not in other tissues including adrenal and ovary. A single 4.0 kb LPL mRNA species was detected in epididymal fat, heart, psoas muscle, lactating mammary gland, adrenal, lung, and ovary, but not in adult kidney, liver, intestine, or brain. Quantitative slot-blot hybridization analysis demonstrated the following relative amounts of LPL mRNA in rat tissues: adipose, 100%; heart, 94%; adrenal, 6.6%; muscle, 3.8%; lung, 3.0%; kidney, 0%; adult liver, 0%. The same quantitative analysis was used to study lipase mRNA levels during development. There was little postnatal variation in LPL mRNA in adipose tissue; maximal levels were detected at the earliest time points studied for both inguinal and epididymal fat. In heart, however, LPL mRNA was detected at low levels 6 days before birth and increased 278-fold as the animals grew to adulthood. Levels of LPL mRNA in lung, psoas muscle, and adrenal gland and levels of HL mRNA in liver showed the same biphasic pattern during development: a 2.4- to 11.3-fold increase around the time of birth followed by a 2.3- to 19.9-fold increase at weaning. Thus, developmental regulation of the genes for two different lipases, HL (in liver) and LPL (in several tissues), may be similar.—**Semenkovich, C. F., S-H. Chen, M. Wims, C-C. Luo, W-H. Li, and L. Chan.** Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation, and evolution. *J. Lipid Res.* 1989. **30:** 423–431.

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Triacylglycerols constitute a major component of circulating lipoprotein particles. While the role of triacylglycerols in the pathogenesis of atherosclerosis is uncertain, recent epidemiologic evidence suggests that elevated triacylglycerols may mediate myocardial infarction risk through effects on fibrinolytic activity (1, 2). Lipoprotein lipase (LPL) and hepatic lipase (HL) are distinct enzymes that hydrolyze lipoprotein triacylglycerols. LPL is essential for normal chylomicron and very low density lipoprotein catabolism and the transfer of cholesterol, phospholipids, and apolipoproteins between lipoprotein particles (3, 4). HL may also be important in lipoprotein and phospholipid metabolism (5, 6), and, through its effects on high density lipoproteins, may mediate the delivery of cholesterol from peripheral tissues to the liver (7–9).

Regulation of HL has not been extensively studied. LPL regulation is complex and shows tissue specificity, e.g., heart and adipose tissue respond differently to the same physiologic and hormonal signals (10, 11). Several studies (12–16) have reported changes in LPL during development, but were limited to enzyme activity measurements. Recently, the sequences of LPL cDNAs from mouse (17), human (18), bovine (19), and guinea pig (20), and HL cDNAs from rat (21) and human (22, 23) have been reported. We now report the characterization of a complete mouse LPL cDNA clone. Using the complete coding sequence of LPL mRNAs from four species, we analyzed the rate of nucleotide and amino acid substitutions from different regions of the enzyme, information useful for future structure-function studies of LPL. Further, we used the complete mouse LPL cDNA and rat HL cDNA to study tissue distribution and developmental regulation of lipase mRNA in the rat.

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; cDNA, complementary DNA; mRNA, messenger RNA; kb, kilobase(s).

MATERIALS AND METHODS

cDNA cloning and sequence analysis

A mouse macrophage cDNA library in λgt11 (Clontech Laboratories, Inc.) was screened by oligonucleotide hybridization using published DNA sequence from a partial mouse cDNA (17). Approximately 2×10^5 recombinants were screened and five mLPL cDNA clones were sequenced by the method of Sanger, Nicklen, and Coulson (24) after subcloning in M13 vectors mp18 and mp19. Rat HL cDNA cloning was performed in our laboratory by oligonucleotide hybridization. The clone contained the complete insert including all the coding and 3' untranslated regions as well as 16 bases in the 5' untranslated region (23). A cloned Chinese hamster β-tubulin cDNA insert (25) was graciously provided by Drs. B. Boggs and F. Cabral. A plasmid containing a human fibroblast β-actin cDNA (pHFβA-3'UT) (26) was a gift from Dr. L. Kedes.

Animals

Sprague-Dawley rats (males at various ages and timed-pregnant females) were purchased from Harlan-Sprague-Dawley, Houston, TX. Animals were housed in an animal facility with a 14 hr/10 hr light/dark cycle and given free access to chow and water for several days before manipulation. Pregnant females were kept in separate cages; birth occurred on day 21 of gestation which was defined as day 1 for purposes of this study. Litter size varied between 9 and 15. Nursing animals were weaned on day 21 of life. Rats were killed between the hours of 10 and 11 AM to ensure that diurnal variation in LPL activity was not a factor (27). All rats were studied in the fed state, verified by the presence of milk in the stomach of nursing rats and chow in the stomach of weaned rats. Fetal and early postnatal tissues were identified with a Wild (Heerbrugg, Switzerland) dissecting microscope under 6.5–10× magnification, and all nonadipose tissues were carefully dissected free of fat.

RNA preparation and analysis

RNA was prepared as described by Chirgwin et al. (28) except some adipose tissue samples were prepared as described by Han, Stratowa, and Rutter (29). Poly(A) RNA was prepared as described by Aviv and Leder (30). For Northern blot analysis, total or poly(A) RNA was electrophoresed in 1.5% agarose/7% formaldehyde gels and transferred to Zeta-Probe membranes (Bio-Rad, Richmond, CA). For slot-blot analysis, total RNA was denatured (31) just before blotting. Samples containing 0.1–20 μg of total RNA were blotted onto Zeta-Probe membranes using a Minifold II apparatus (Schleicher and Schuell) and baked at 80°C for 2 hr in a vacuum oven. mLPL-1 (1.8 kB) and rHL-2 (1.6 kB) were labeled with ^{32}P-αdNTP by nick-translation or random oligonucleotide priming for Northern analysis and by nick-translation for all slot-blot analysis. cDNAs were consistently labeled to a specific activity of approximately 5×10^8 cpm/μg DNA. Each slot-blot asssay included as control at least one slot containing 10 μg of total yeast RNA that did not hybridize to mLPL-1 or rHL-2. Conditions of prehybridization and hybridization were essentially as previously described (31) except that blots were exposed to film and stored wet to allow stripping and rehybridization. Because of the low abundance of lipase mRNA in several tissues, blots were exposed to film in the presence of two intensifying screens for up to 2 weeks at −70°C. Autoradiograms were scanned with a MacBeth TD932 densitometer. Actin and tubulin blots were used as controls; lipase signals were not normalized to the control signals.

Estimation of the number of nucleotide substitutions

The method of Li, Wu, and Luo (32) was used. In this method, nucleotide sites and substitutions are classified as synonymous (causing no amino acid change) and nonsynonymous. For example, the first two positions of the codon UUU are nonsynonymous, while the third position is counted as one-third synonymous and two-thirds nonsynonymous. The method gives the number (K_S) of (synonymous) substitutions per synonymous site and the number (K_A) of (nonsynonymous) substitutions per nonsynonymous site.

RESULTS AND DISCUSSION

Complete sequence of cloned mouse LPL cDNA

We have sequenced a complete mouse LPL cDNA. The sequence of the mature peptide and 3' untranslated regions is identical to the sequence reported previously by Kirchgessner et al. (17). These authors presented a partial mouse LPL signal peptide sequence from residues −19 to −1. The 5' untranslated region and the nucleotide sequence for the eight amino-terminal residues (−27 to −20), unavailable in the previous study, are presented in **Fig. 1A.**

Evolution of LPL genes

Table 1 shows the number (K_S) of substitutions per synonymous site and the number (K_A) of substitutions per nonsynonymous site between each pair of genes. The bovine lineage separated from the other lineages about 80 million years ago, the human lineage separated from the rodent lineage about 75 million years ago, and the mouse and guinea pig lineages diverged about 60 million years ago (33). In comparing the rates of evolution in mouse

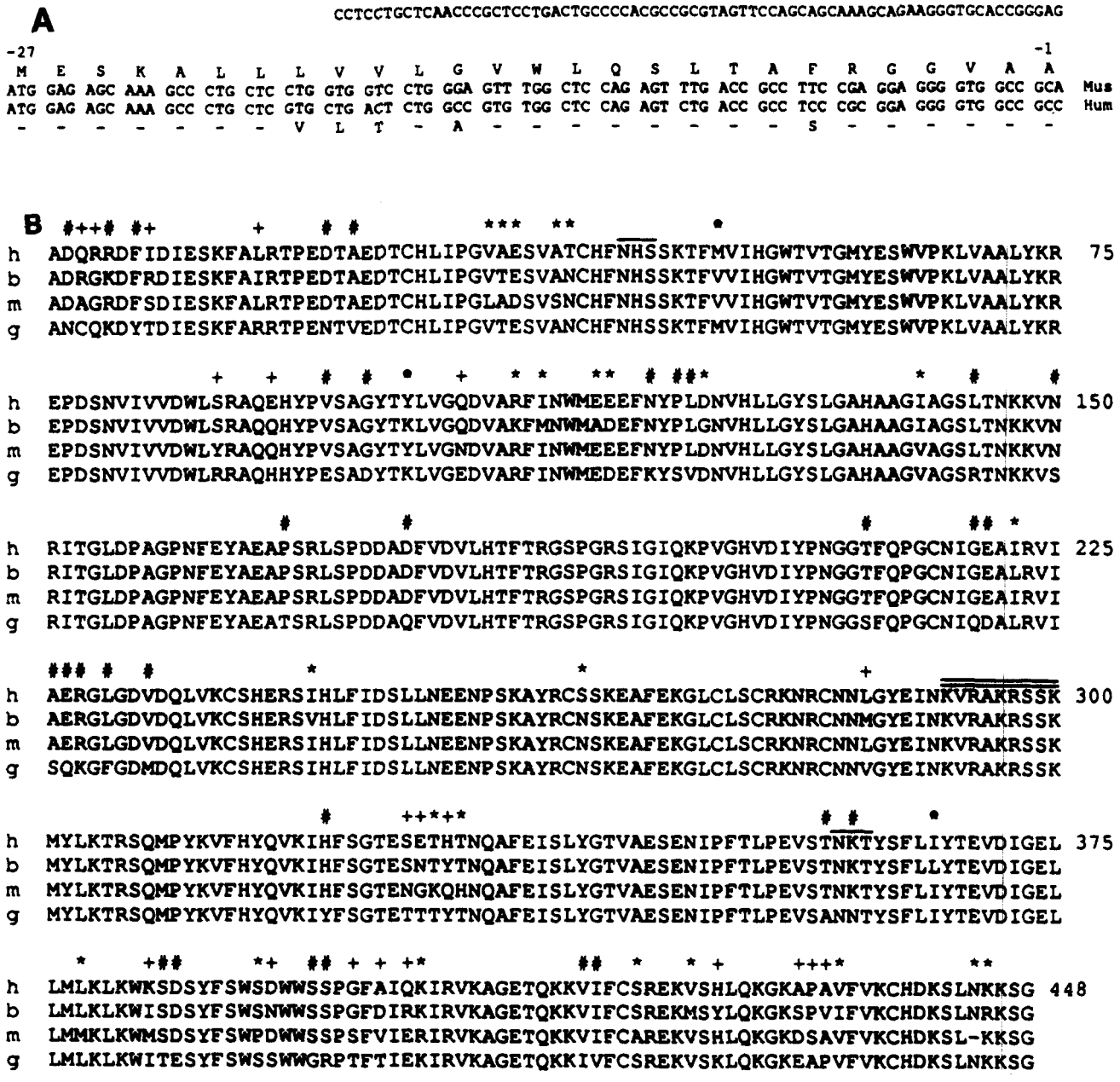

Fig. 1. (A) Sequence of 5' untranslated and signal peptide regions of mouse LPL mRNA. The signal peptide region is aligned with the human sequence; identical amino acid residues are marked by -. The bovine, but not the guinea pig, LPL signal peptide sequence can also be readily aligned without the introduction of gaps. (B) Alignment of human (h), bovine (b), mouse (m), and guinea pig (g) LPL. For the sources of sequence data, see text. Among the four sequences, only one deletion (in mouse; residue 444) is observed. Positions where the residues are identical in the human, bovine, and mouse LPLs, but different in the guinea pig LPL are marked by #. Positions where two types of amino acids are observed among the four sequences are marked by *, and positions where more than two types of amino acids are observed are marked by +. The potential N-glycosylation sites are marked by single bars and the putative heparin-binding site is marked by double bars.

and guinea pig genes, we use bovine and human genes as outgroup references. The K_S value between human and mouse is lower than that between human and guinea pig, but the difference is not statistically significant. The K_S value between bovine and mouse is higher than that between bovine and guinea pig, but the difference is also not significant. From these two comparisons, we assume that the rate of synonymous substitution is the same in the mouse and guinea pig lineages. The K_S value between the two species is 0.677, so the rate of synonymous substitution is $0.677/(2 \times 60 \times 10^6) = 5.64 \times 10^{-9}$ substitutions/site per year. Using bovine as a reference and following the method used in Li, Tanimura, and Sharp (34), we estimate that since the divergence between the human and rodent lineages, the rate of synonymous substitution is 5.80×10^{-9} in the rodent lineage, which is close to the

TABLE 1. Number of substitutions per synonymous site (above diagonal) and number of substitutions per nonsynonymous site between genes in the region coding for LPL

	Human	Bovine	Mouse	Guinea Pig
Human		0.549 ± 0.060	0.607 ± 0.087	0.673 ± 0.071
Bovine	0.033 ± 0.006		0.816 ± 0.087	0.737 ± 0.080
Mouse	0.032 ± 0.006	0.046 ± 0.007		0.677 ± 0.071
Guinea pig	0.071 ± 0.008	0.071 ± 0.009	0.077 ± 0.009	

above estimate, and 2.73×10^{-9} in the human lineage. We also estimate that the rate of synonymous substitution is 4.05×10^{-9} in the bovine lineage. Thus, the synonymous rate is highest in the rodent lineage, and lowest in the human lineage. These results are consistent with those from other genes (34).

Considering the rate of nonsynonymous substitution, we found the K_A value (0.071) between human and guinea pig significantly higher than that (0.032) between human and mouse. The K_A value (0.071) between bovine and guinea pig is also significantly higher than that (0.046) between bovine and mouse. Using both bovine and human as references and following the method used in Li et al. (34), we estimate that the rate of nonsynonymous substitution is 0.90×10^{-9} in the guinea pig lineage and 0.33×10^{-9} in the mouse lineage. We also estimate that the rate of nonsynonymous substitution is 0.16×10^{-9} in the human lineage and 0.21×10^{-9} in the bovine lineage. It is not clear why the rate of nonsynonymous substitution is much higher in the guinea pig than in the other lineages. Moreover, the signal peptide sequence of the guinea pig LPL cannot be reliably aligned with those of the other three LPLs, though the latter can be easily aligned (see Fig. 1A).

Fig. 1B shows an alignment of human, bovine, mouse, and guinea pig LPL sequences. Nonconserved residue sites are marked by +, *, and #, which signify, respectively, that at the site, more than one amino acid change has occurred among the four sequences, that an amino acid change has occurred in one of the human, bovine and mouse sequences, and that an amino acid change has occurred only in the guinea pig sequence. There are, in total, 31 #s, 27 *s, and 20 +s; the large number of #s reflects the fact that the guinea pig sequence has evolved much faster than the other sequences. The amino acid changes tend to be clustered, occurring mainly in the following regions: residues 2–40, 88–134, 211–233, 321–331, and 358–445. The proportion of residues conserved in all four sequences is 370/448 = 83%. The two largest segments that contain no change are residues 178–210 and 287–320; the latter encompasses the putative heparin-binding site (20). Two potential N-glycosylation sites are conserved in all four sequences, though in the second site, an amino acid change has occurred in the guinea pig sequence.

Distribution of HL and LPL mRNA in adult rat tissues

Northern blot analysis of adult rat tissue RNA using ^{32}P-labeled rHL-2 as probe demonstrated the presence of an ~1.87 kb HL mRNA in liver only (**Fig. 2A,** lane 6). No HL mRNA was seen in other tissues including adrenal gland (lane 1) despite prolonged exposures. In other blots (not shown), HL mRNA was detected in liver poly(A) RNA (8 μg), but not in adult ovary poly(A) RNA (16 μg) even after prolonged exposure. These findings confirm those of Komaromy and Schotz (21) and extend their results by providing Northern blot analysis of six additional tissues (pancreas, adipose tissue, lung, muscle, mammary gland, and ovary). Others have detected HL-like activity or protein in rat adrenals and ovaries (35, 36). Doolittle et al. (37) recently showed that rat adrenal is unable to synthesize HL, and suggested that HL synthesized in liver may be transported to extrahepatic sites such as adrenal and ovary. Our work supports this hypothesis.

A single LPL mRNA species was detected in a number of adult rat tissues (Fig. 2B). Rat appears to be unique in this regard since mouse (17), human (18), and bovine (19) LPL mRNAs consist of two or three different-sized species. Rat epididymal fat, heart, psoas muscle, and lactating mammary gland contained LPL mRNA of ~4.0 kb on Northern analysis. The overexposed blot of lanes 6–12 shows the presence of the same LPL mRNA in adrenal and lung as well as a faint, intact LPL mRNA in adult ovary (lane 8). Therefore, ovary appears to have the capacity to synthesize LPL but not HL. No LPL mRNA was detected in adult kidney, liver, intestine, or brain, even on prolonged exposure. LPL-like activity has been reported in rat brain, but is very low compared to other tissues (12, 38). Since low concentrations of LPL mRNA have been demonstrated in mouse brain (17), it is possible that the level of LPL mRNA in the rat brain is too low to be detected by blot analysis, or that there is a species-specific difference between rat and mouse. We also observed other differences in the tissue distribution of LPL mRNA among species. For example, LPL mRNA has been detected in mouse liver (17), but not in human liver or HepG2 cells (18), or adult rat liver (present study). LPL mRNA has also been detected in mouse (17) and

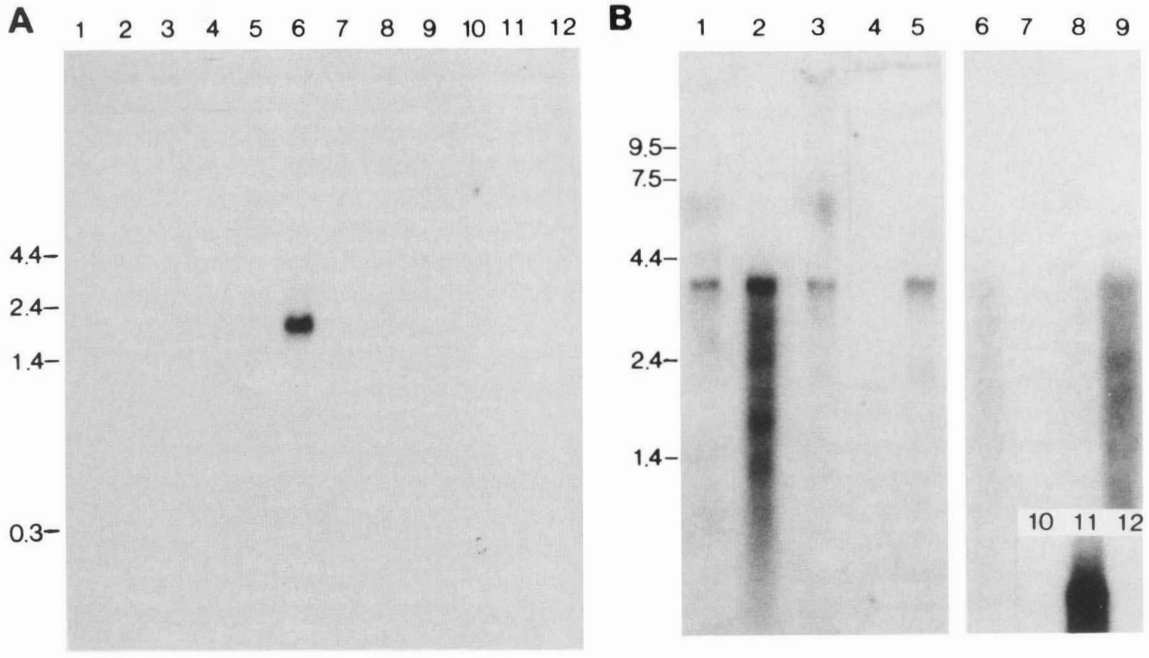

Fig. 2. Northern blot analysis of HL and LPL mRNA. (A) Northern blot hybridization of ^{32}P-labeled rHL-2 to RNA from rat tissues. Each lane contained 3–9 μg poly(A) RNA except lane 12 which contained 10 μg total RNA from lactating mammary gland. Lane 1, adrenal; lane 2, heart; lane 3, epididymal fat pad; lane 4, testis; lane 5, brain; lane 6, liver; lane 7, intestine; lane 8, lung; lane 9, kidney; lane 10, pancreas; lane 11, psoas muscle; lane 12, lactating mammary gland. (B) Northern blot hybridization of ^{32}P-labeled mLPL-1 to RNA from rat tissues. Lane 1, epididymal fat pad, 20 μg total RNA; lane 2, heart, 10 μg poly(A) RNA; lane 3, psoas muscle, 6 μg poly(A) RNA; lane 4, kidney, 6 μg poly(A) RNA; lane 5, lactating mammary gland, 10 μg total RNA. Lanes 6–12 are from a blot that was overexposed to allow identification of the faint, intact band in lane 8 and to emphasize the lack of signal in lanes 7, 10, and 12. Each lane contained poly(A) RNA. Lane 6, adrenal (10 μg); lane 7, liver (10 μg); lane 8, ovary (10 μg); lane 9, lung (20 μg); lane 10, intestine (10 μg); lane 11, heart (10 μg); lane 12, brain (20 μg).

human (18) kidney, but not in kidney from guinea pig (20) or rat (present study).

Since the relative concentrations of LPL mRNA in tissues have never been quantified in any species, we examined this problem in the rat by quantitative slot-blot hybridization (**Fig. 3**). With this technique, the intensities of signals produced by hybridization of ^{32}P-labeled LPL cDNA to increasing amounts of total RNA were determined and plotted (see Fig. 4 below). The regression coefficients (slopes) of these linear plots were used to determine the tissue concentrations of LPL mRNA. LPL mRNA was most abundant in adipose tissue (epididymal fat pad) and heart. Substantially lower levels of LPL mRNA were present in adrenal gland, muscle, and lung (Fig. 3). No LPL mRNA signal was detected by slot-blot analysis in adult liver (see below) or kidney.

Expression of LPL and HL mRNA in rat tissues during development

Developmental regulation of LPL and HL mRNA was studied by quantitative slot-blot analysis. RNA was prepared from various tissues on days −6 (6 days before birth or day 15 of gestation), −4, −2, −1 and days 1 (day of birth), 3, 7, 13, 20, 24, 37, or 40 and from adults (60–100

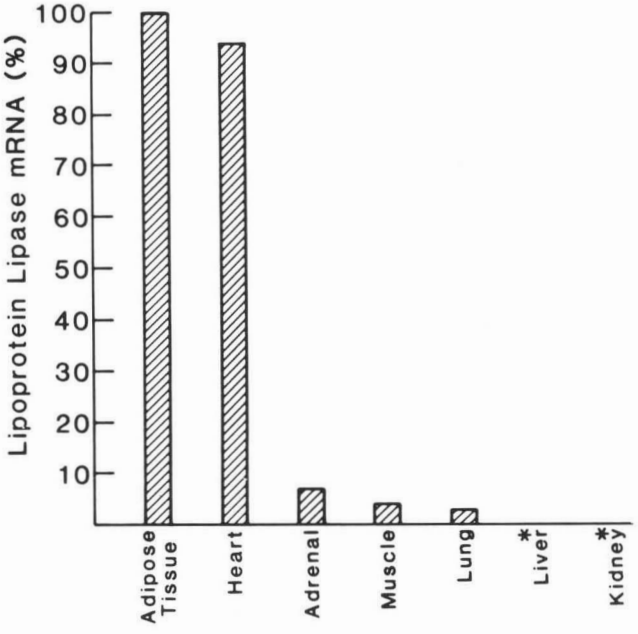

Fig. 3. Concentrations of hybridizable LPL mRNA sequences in rat tissues determined by quantitative slot-blot analysis. Total RNA from adult animals was assayed by hybridization to ^{32}P-labeled mLPL-1 as described in the legend to Fig. 4; *, none detected.

days of age). Representative plots of slot-blots for adipose tissue, heart, and muscle for LPL mRNA and liver for HL mRNA at different stages of development are shown in **Fig. 4.** Similar linear plots were obtained for adrenal gland and lung during development. To ensure that variations in mRNA were not due to differences in RNA content or quality, all developmental slot-blots were reanalyzed with control cDNAs. Adipose and adrenal slot-blots were reprobed with β-actin cDNA; there was minimal variation in β-actin-mRNA levels during development. Heart, lung, muscle, and liver were reprobed with β-tubulin cDNA which showed the same pattern in all tissues: β-tubulin mRNA levels were unchanged between days −6 and 3 and decreased thereafter.

LPL mRNA expression in adipose tissue during development is shown in panel A of **Fig. 5.** Adipose tissue has not been found in prenatal rats and is difficult to detect in the early postnatal period. Postnatally, adipose cells first accumulate in the inguinal region, then regress at this depot while other regions of fat, including the epididymal fat pad, increase in size. A level of LPL mRNA similar to that of adult epididymal fat pad was detected in inguinal fat on day 3. The mRNA levels decreased in concert with the decrease in size of this depot, a pattern similar to that reported for LPL activity in inguinal fat by Chajek, Stein, and Stein (12). Epididymal fat LPL mRNA levels were high at the first time point studied (day 24), and did not vary subsequently. Hietanen and Greenwood (13) have reported that LPL activity (expressed per mg of protein) in developing epididymal fat essentially does not change after day 21, a pattern identical to that seen for LPL mRNA levels in the current study. Chajek and colleagues (12) however, measured a significant increase in LPL activity after day 20 followed by a fall in activity to adult levels. The reason for this discrepancy is unclear.

Unlike adipose tissue, rat heart is formed relatively early in gestation. LPL mRNA was detected at low levels in rat heart 6 days before birth (Fig. 5B) and increased 13.9-fold on day 1. The levels further increased during development with peak LPL mRNA expression detected in adult heart at levels 278-fold greater than those detected 6 days before birth. Studying LPL enzyme activity, Chajek et al. (12) reported a similar pattern of induction of LPL in developing rat heart and reported that cardiac LPL induction was associated with the development of

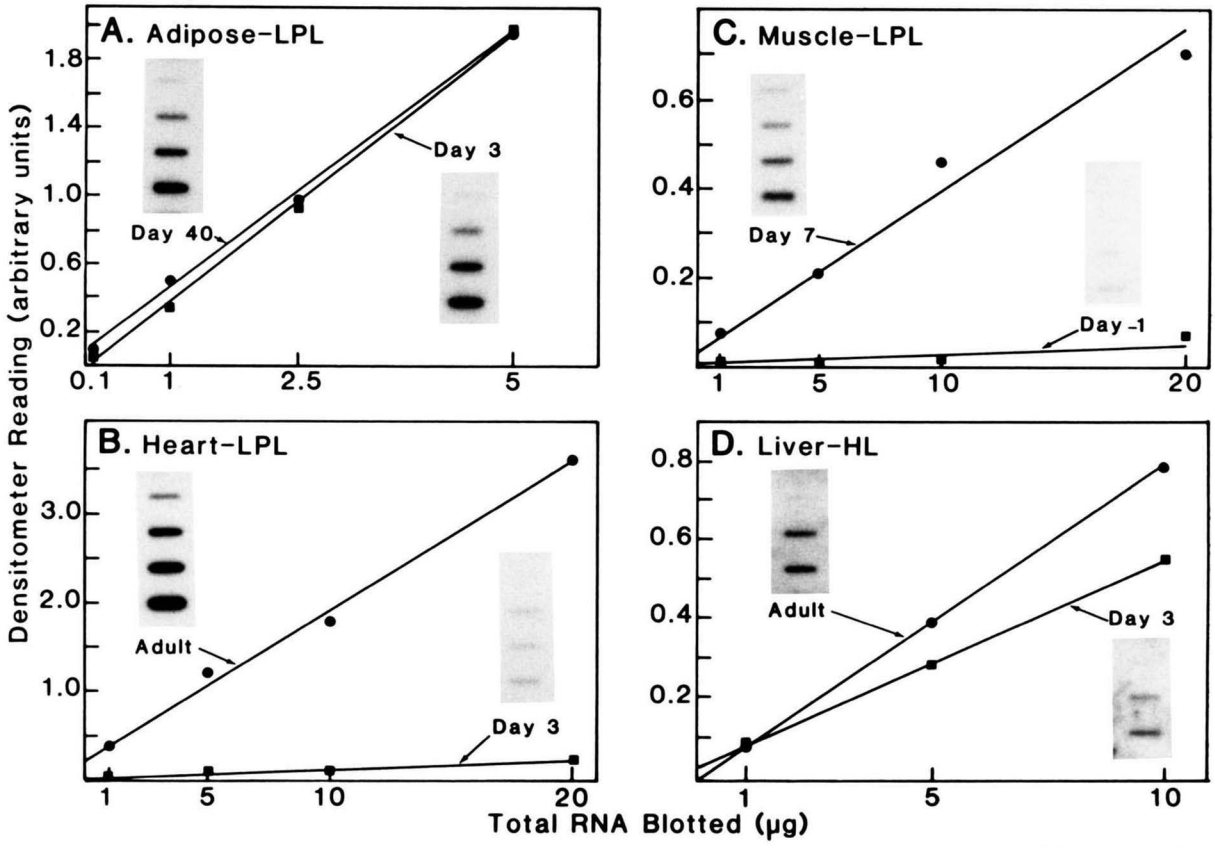

Fig. 4. Slot-blot analysis of lipase mRNA. Various concentrations of total RNA from several tissues were blotted and hybridized to ^{32}P-labeled mLPL-1 (panels A-C) or ^{32}P-labeled rHL-2 (panel D). Filters were exposed to X-ray film, autoradiograms (insets) were scanned, and the results were plotted. Data are shown for two representative time points during development for four tissues. Similar linear plots were generated at each time point during development in each tissue studied.

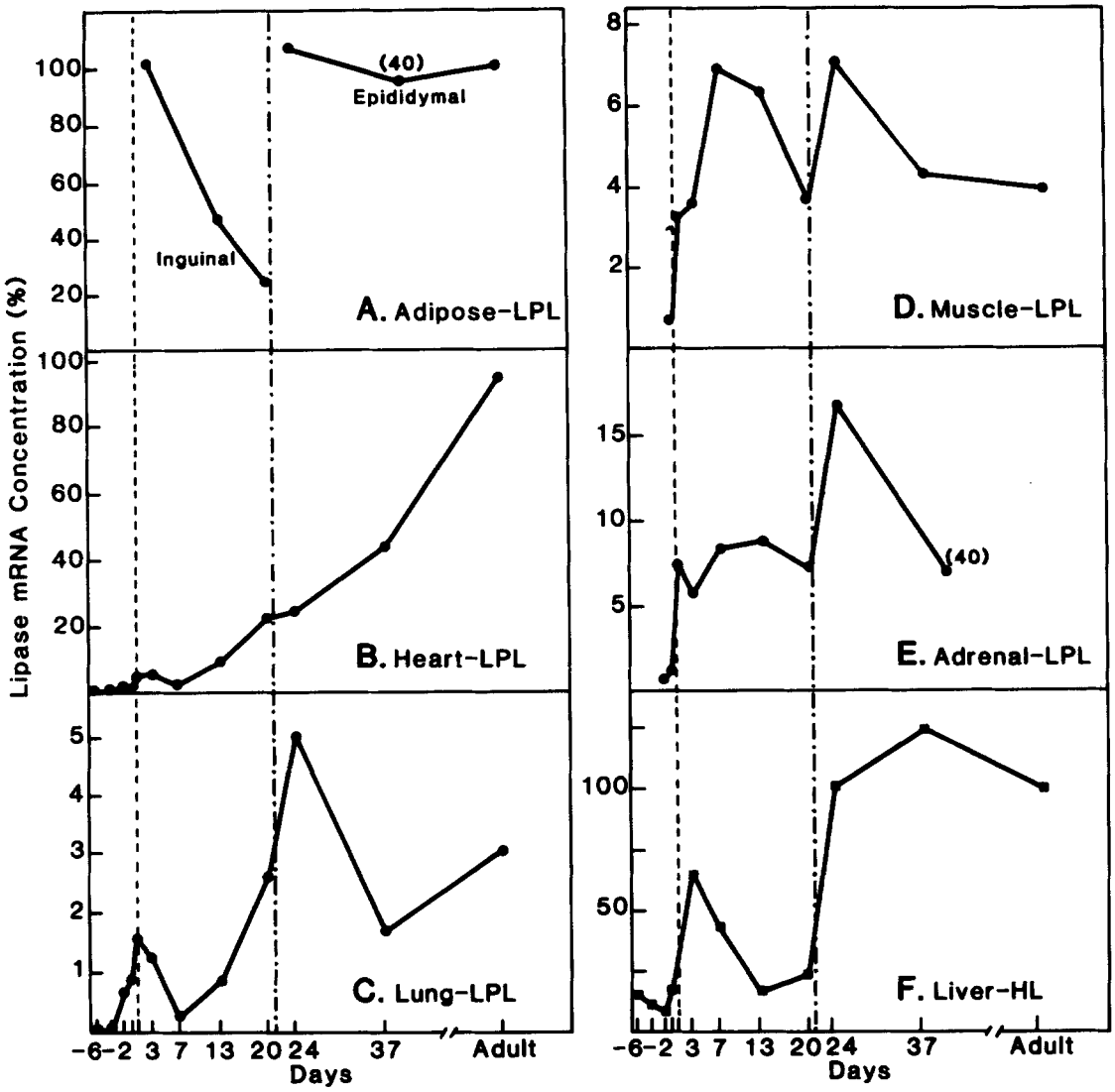

Fig. 5. Relative concentrations of lipase mRNA during development. Concentrations of LPL mRNA (panels A-E) and HL mRNA (panel F) were determined from the slopes of linear plots of slot-blot experiments (Fig. 4). All LPL mRNA concentrations were normalized to the concentration of LPL mRNA in adult epididymal fat pad (100%). For HL, mRNA concentrations were determined relative to the concentration of HL mRNA in adult liver (100%); (--) day of birth; (-· -) day of weaning.

myocardial capillaries. Since LPL acts at the capillary endothelial surface, these authors speculated that capillary development may effect LPL induction. Our work indicates that the unidentified molecular signal for LPL induction acts at the mRNA level.

LPL mRNA in lung (Fig. 5C) was first detected 2 days before birth. Levels increased 2.4-fold at birth, followed by a decline and a subsequent 19.9-fold increase between days 7 and 24. Psoas muscle (Fig. 5D) contained detectable concentrations of LPL mRNA 1 day before birth which increased 10-fold by day 7 of life. Muscle LPL mRNA increased 2.3-fold between days 20 and 24, time points separated by a change from an essentially all-fat diet to a chow diet. A similar pattern was seen in adrenal gland (Fig. 5E) where an 11.3-fold increase in LPL mRNA occurred between days −1 and 1 and another 2.4-fold increase occurred between days 20 and 24. Other investigators detected increases in LPL activity in developing rat lung and skeletal muscle at birth (12, 15). At least one group reported a subsequent increase in LPL activity in rat lung around the time of weaning (12). The current work suggests that changes in mRNA levels play a major role in mediating developmental changes in LPL activity.

Newborn rat liver has been reported to contain LPL-like activity at a level 19 to 57% of that in adult fat and heart (39). LPL mRNA was not detected in rat liver prenatally or on day 1, even on prolonged exposure. However, extremely low but detectable levels of LPL-like mRNA were observed beginning on day 3 in the same pattern seen for LPL in several tissues. The relative amounts of

LPL-like mRNA compared with adult adipose RNA samples on the same blot were: adipose, 100%; prenatal and day 1 liver (five samples), 0.00%; day 3, 0.49%; day 7, 0.19%; day 13, 0.05%; day 20, 0.00%; day 24, 0.44%; day 37 and adult, 0.00%. Northern blot analysis of these RNA samples failed to show a discrete band of LPL mRNA, possibly because of its extremely low concentration. The significance of the very low amounts of LPL mRNA-like material detected by slot-blot analysis is unclear. Even if it were authentic LPL mRNA, it cannot account for the substantial LPL-like activity detected in neonatal rat liver (39), which represents either a different enzyme activity or LPL originating from another site and anchored on the hepatic capillary endothelium.

HL mRNA was detected in liver 6 days before birth at a level 15% of that in adult liver (Fig. 5F). Others have detected HL activity 6 days before birth at 31% of that in adult liver (12). Despite the fact that HL is a different enzyme than LPL, HL mRNA levels in developing rat liver showed a pattern remarkably similar to that seen for LPL mRNA in some other tissues. HL mRNA increased 7.9-fold at the time of birth and 7.5-fold between days 13 and 24. Previous studies (17, 21-23) suggested that HL and LPL share similar structure and function and may be members of a multigene family. Our work suggests that the genes for these two enzymes also show similar patterns of developmental regulation. We speculate that insulin might play a role in such regulation since rat insulin levels are known to peak at birth and at weaning (40), coincident with peaks in lipase mRNA levels in a number of tissues (Fig. 5). Recent preliminary studies suggest that, at least in cultured rat adipocytes, insulin in physiologic concentrations is capable of increasing LPL mRNA (41, 42).

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