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Induced mutant mouse lines that express lipoprotein lipase in cardiac muscle, but not in skeletal muscle and adipose tissue, have normal plasma triglyceride and high-density lipoprotein-cholesterol levels

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ABSTRACT **The tissue-specific expression of lipoprotein lipase (LPL) in adipose tissue (AT), skeletal muscle (SM), and cardiac muscle (CM) is rate-limiting for the uptake of triglyceride (TG)-derived free fatty acids and decisive in the regulation of energy balance and lipoprotein metabolism. To investigate the tissue-specific metabolic effects of LPL, three independent transgenic mouse lines were established that expressed a human LPL (hLPL) minigene predominantly in CM. Through cross-breeding with heterozygous LPL knockout mice, animals were generated that produced hLPL mRNA and enzyme activity in CM but lacked the enzyme in SM and AT because of the absence of the endogenous mouse LPL gene (L0-hLPL). LPL activity in CM and postheparin plasma of L0-hLPL mice was reduced by 34% and 60%, respectively, compared with control mice. This reduced LPL expression was sufficient to rescue LPL knockout mice from neonatal death. L0-hLPL animals developed normally with regard to body weight and body-mass composition. Plasma TG levels in L0-hLPL animals were increased up to 10-fold during the suckling period but normalized after weaning and decreased in adult animals. L0-hLPL mice had normal plasma high-density lipoprotein (HDL)-cholesterol levels, indicating that LPL expression in CM alone was sufficient to allow for normal HDL production. The absence of LPL in SM and AT did not cause detectable morphological or histopathological changes in these tissues. However, the lipid composition in AT and SM exhibited a marked decrease in polyunsaturated fatty acids. From this genetic model of LPL deficiency in SM and AT, it can be concluded that CM-specific LPL expression is a major determinant in the regulation of plasma TG and HDL-cholesterol levels.**

The catabolism of triglycerides (TGs) in postprandial chylomicrons and hepatically synthesized very low density lipoproteins (VLDLs) in extrahepatic tissues is mediated principally by lipoprotein lipase (LPL; see refs. 1 and 2 for review). TG hydrolysis results in the generation of large amounts of free fatty acids (FFA) that are taken up by adipocytes for storage and by muscles for oxidation. Accordingly, the enzyme is vital to energy homeostasis as well as the metabolism of plasma lipoproteins (3). The highest LPL activities are observed in adipose tissue (AT), skeletal muscle (SM), and cardiac muscle (CM), where the enzyme is bound to glucosaminoglycans at the luminal side of the capillary endothelium. This local tethering of the enzyme to the tissues with the highest demand for FFA, together with the large variation in enzyme expression in AT, SM, and CM in response to nutritional and hormonal changes, confers a regulatory function to LPL for the partitioning of FFA among these tissues. This ability of LPL to direct the flux of FFA in the body suggested a "gate-keeping hypothesis," which implicated LPL as the determining factor for the amount of FFA taken up in AT compared with the amount taken up in muscle (4). It follows that balanced regulation of LPL activities in these tissues might be crucial to maintain normal body weight.

The impact of LPL on the metabolism of plasma lipoproteins is well documented (3, 5). LPL deficiency, as observed in patients with type I hyperlipoproteinemia, causes high TG and low high-density lipoprotein (HDL)-cholesterol levels in plasma, whereas high LPL activities are associated with low plasma TG and elevated HDL-cholesterol levels (6, 7). According to current models, the negative correlation between plasma TG levels and HDL-cholesterol results from the subsequent commitment of the products of the hydrolysis of TG-rich lipoproteins as precursors to the synthesis of HDL particles. Therefore, it is generally believed that the total amount of endothelial cell-associated LPL in the vascular system, in addition to the hepatic synthesis of TG-rich lipoproteins, determines the plasma concentration of TG and HDL-cholesterol (8). The effect of variation in LPL expression in specific tissues on the metabolism of plasma lipids and lipoproteins is largely unknown.

To elucidate the tissue-specific effects of LPL expression on AT development, body-mass composition, and the metabolism of plasma lipoproteins, induced mutant mouse lines were generated that lack LPL (9, 10) or that overexpress the enzyme predominantly in SM (11–14). For this study, transgenic mouse lines were established that expressed a human LPL (hLPL) minigene under the control of 8 kilobases (kb) of 5′ flanking region of the mouse LPL gene. The transgene was expressed predominantly in CM. No hLPL mRNA or enzyme activity was observed in SM or AT. These transgenic mouse lines were crossbred with heterozygous LPL knockout (L1) mice to generate a unique animal model that expressed LPL exclusively in CM in an otherwise essentially LPL-deficient animal.

METHODS

LPL-Transgene Construction. To study the effect of upstream regulatory sequences of the LPL gene on its tissue-specific expression pattern, 8 kb of 5′ flanking region of the mouse LPL gene were fused with an LPL minigene, and this DNA-construction was used for the generation of transgenic mouse lines. For the cloning procedure, three overlapping mouse genomic clones, extending up to −16 kb upstream from the 5′ end

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Abbreviations: AT, adipose tissue; CM, cardiac muscle; FFA, free fatty acids; HDL, high-density lipoprotein; hLPL, human LPL; kb, kilobases; L0, LPL knockout mice; L1, heterozygous knockout mice; L2, wild-type mice; LPL, lipoprotein lipase; PHP, post heparin plasma; PUFA, polyunsaturated fatty acids; SM, skeletal muscle; TG, triglycerides; VLDL, very low density lipoprotein.

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of the transcriptional start site, were used. A DNA fragment from phage clone λ10, spanning from −4.6 kb to −0.6 kb upstream of the transcriptional start site, was ligated with an *Eco*RI–*Spe*I fragment spanning from −0.6 kb to +36 bp within the first intron of the gene. This 4.6-kb piece of DNA subsequently was fused with the 5.0-kb piece of DNA containing a hLPL minigene that has been described and used in several studies (11, 13, 14). The resulting construction was cut with *Sac*I and ligated to a 5.7-kb *Sac*I restriction fragment (from phage clone λ92) spanning from −8 kb to −2.4 kb. Accordingly, the complete DNA construction for microinjection contained 8 kb of the 5′ flanking region of the mouse LPL gene fused to the hLPL minigene and was named *hlpl*. Before microinjection, *hlpl*-DNA was prepared by using Qiaex (Qiagen, Chatsworth, CA) and subsequently was purified by an Elutip D column obtained from Schleicher & Schuell.

Generation and Screening of Transgenic Mice on the Wild-Type Background. Transgenic mice were created as described (11). *hlpl*-DNA was injected into the fertilized pronuclei of (C57BL/6J × CBA/J)F_1 females that had been mated to males of the same genetic background. Founder animals were bred to (C57BL/6J × CBA/J)F_1 animals, and three transgenic lines were established with the hlpl construction: L2-hLPLA, L2-hLPLB, and L2-hLPLC. Integration of the hLPL minigene in the founder animals and their progeny was determined by Southern blotting or PCR. Tail-derived DNA (10 μg) was digested with *Pvu*II, fractionated by agarose gel electrophoresis, and blotted onto nylon membrane. DNA corresponding to a hLPL minigene was detected with a radiolabeled 1.2-kb *Nco*I–*Eco*RI fragment from the hLPL cDNA. Nontransgenic littermates were used as controls. A rapid PCR analysis to screen for the presence of the minigene was performed as described (13).

Generation and Screening of Transgenic Mice on the L1 Background. L2-hLPLB transgenic mice were cross-bred with heterozygous L1 mice (9). L1 mice were prepared in the (C57BL/6J × 129/J) background and maintained in the heterozygous state, because homozygosity (L0) was lethal. F_1 mice, which were hemizygous for the hLPL transgene and heterozygous for the knockout mutation, were again crossed to L1 mice. Some of the F_2 progeny (12.5%) should lack mouse LPL and be hemizygous for the hLPL transgene (L0-hLPL). These mice were genetic hybrids of strains C57BL/6J, CBA, and 129/J. For the screening of these mice, tail-tip DNA was subjected to two PCRs. One PCR was performed to detect the hLPL transgene, and the second was performed to screen for the presence or absence of the knockout mutation by using a protocol exactly as described (13).

RNA Analysis. By using acid guanidinium thiocyanate-phenol chloroform extraction, RNA was prepared from various tissues of adult control and transgenic mice and from CM of neonates (4, 11, 18, and 24 days after birth; ref. 15). Northern blotting analysis was performed as described (11). To detect mouse LPL mRNA, a 0.7-kb *Pst*I fragment from exon 10 of the mouse LPL gene was used as a probe. To differentiate hLPL mRNA from the endogenous mouse mRNA, a species-specific DNA-probe containing a 1-kb *Eco*RI fragment from exon 10 of the hLPL gene was used (11).

Lipolytic Enzymes, Growth Curves, and Body-Mass Composition. These parameters were determined as described (11, 13, 14). LPL activity was determined in postheparin plasma (PHP) and tissue specimens from mice having free access to food (fed) and mice that had fasted for 8 h (fasted). For growth analyses, male mice of each group were maintained on a chow diet (4.5% fat) and were weighed once per week from week 3 (weaning) through week 8. The body-mass composition was deduced from gravimetric determinations of total weight, dry weight, and lipid content.

Lipid, Lipoprotein, and FFA Analysis. Control and transgenic animals were fed regular mouse chow (4.5% fat; 21% protein). Blood was taken in the morning after the animals had free access to food (fed samples) and in the evening after they had fasted 8 h during the day (fasted samples). Plasma levels of HDL-cholesterol, TG, and FFA were determined with commercially available kits. The fatty acid composition of the CM, SM, and epididymal AT were performed as described (14).

***In Vivo* Removal of Labeled VLDL.** To determine VLDL turnover, a protocol used in earlier studies was employed (9, 13). VLDL was isolated from mouse plasma, labeled *in vivo* with [^{3}H]palmitate, reisolated, and injected into animals of all genotypes. The disappearance of radioactivity was monitored in plasma samples at various time points up to 40 min after the initial injection. VLDL-TG kinetics were analyzed by using a single- and two-pool model, based on a main VLDL pool with the assumption that the remnant pool is derived entirely from the main pool (16).

Microscopy and Cytochemical Analyses. Morphological and histochemical analyses were performed with 3.5- to 5-month-old animals of all genotypes. Fresh muscle specimens were prepared from the upper and lower foreleg as well as the hind leg. In addition, samples from all other organs (e.g., the whole brain in frontal slices, the whole heart in transverse sections, lungs, liver, gastrointestinal tract, spleen, kidney, adrenal gland, and urogenital tract) were analyzed. Light microscopy, histochemical, and ultrastructural analyses were performed as described recently (11).

Statistics. Results are expressed as means ± SD. Student's *t* test was used to calculate statistical significance among groups. Statistical significance was defined as $P < 0.05$.

RESULTS

Generation of LPL Transgenic Mouse Lines. The initial goal of this study was to express a hLPL minigene under the transcriptional control of the mouse LPL promoter in transgenic mice. Therefore, an 8-kb DNA fragment 5′ upstream of the transcriptional start site of the mouse LPL gene was fused with a hLPL minigene that has been described in recent reports (11, 13). A map of the transgene is displayed in Fig. 1*A*. The DNA construction was microinjected into fertilized oocytes, and transgenic mouse lines were generated. There were three mouse lines obtained from three independent founders, and the lines were designated L2-hLPLA, L2-hLPLB, and L2-hLPLC. Semiquantitative Southern blotting indicated a similar copy number of the transgene in all three mouse lines (Fig. 1*A*). As judged from the signal intensity, the copy number ranged between 10 and 15 copies per genome, and the transgene segregated in all lines as an autosomal single gene, consistent with a single genomic integration site.

mRNA Analysis. To study transgene expression, Northern blotting analysis was performed on total RNA samples from various tissues of L2-hLPLB mice (Fig. 1*B*). High levels of mouse LPL mRNA were detected in white, brown, and subcutaneous AT, in CM, in kidney, and in aorta. In contrast, hLPL mRNA of the expected size (3.6 kb) was detected predominantly in CM. Small levels of hLPL mRNA were detected additionally in brain, kidney, and aorta at levels that were less than 10% of those observed in CM. No hLPL mRNA was found in SM and white AT. Identical results were obtained from Northern blotting analyses with L2-hLPLA and L2-hLPLC mice (not shown) suggesting that 8 kb of the 5′ flanking region of the mouse LPL gene is sufficient to promote LPL expression in CM but not in SM and AT. Because the highest level of hLPL mRNA was observed in CM of L2-hLPLB mice, this strain was used for all further breeding experiments (and renamed L2-hLPL).

hLPL mRNA expression in CM also was analyzed during animal development within the first 6 weeks of life and compared with the endogenous mouse LPL mRNA expression pattern. Northern blot analyses were performed on total RNA samples from CM of L2-hLPL mice, and blots were hybridized with species-specific DNA probes (Fig. 1*C*). hLPL mRNA levels exhibited a similar expression pattern compared with mouse LPL mRNA levels. Both were low during the first week of life and then increased sharply during the second week after birth.

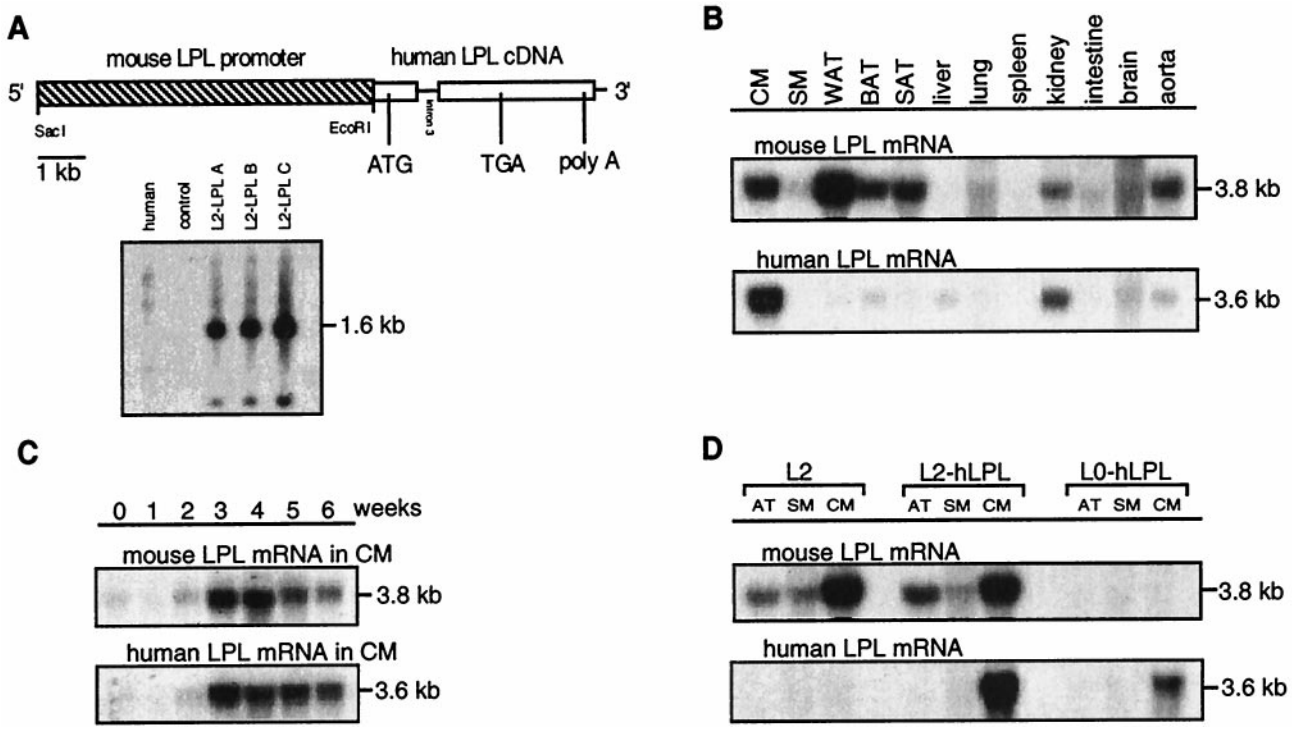

FIG. 1. Map of the transgene DNA construction, DNA analysis, and RNA analysis. (*A*) The LPL minigene DNA construction used for microinjection. The minigene was assembled from hLPL cDNA (open box), the hLPL gene (intron 3 and 3′ sequences), and 8 kb of 5′ flanking sequences of the mouse LPL gene (striped box). The fusion sites between minigene and promoter-sequences, the ATG start codon, the TGA stop codon, and the polyadenylation signal (poly A) are indicated. Southern blotting analysis of tail-tip DNA and hybridization of a radiolabeled probe to a 1.6-kb DNA fragment indicated the integration of the LPL minigene into the mouse genome. DNA isolated from nontransgenic mice or human white blood cells exhibited no specific signal at 1.6 kb. (*B*) Tissue-specific expression pattern of mouse LPL and hLPL mRNA in the indicated tissues and organs of 3.5-month-old L2-hLPL mice. mRNA signals of the appropriate size for mouse LPL (3.8 kb) and hLPL (3.6 kb) are indicated. WAT, BAT, and SAT refer to white, brown, and subcutaneous AT, respectively. (*C*) Induction of mouse LPL and hLPL mRNA in CM of L2-hLPL mice within 6 weeks of birth. (*D*) Mouse LPL and hLPL mRNA expression in AT, SM, and CM of 3.5-month-old male mice (L2-hLPL, L0-hLPL, and control).

Generation of Induced Mutant Mice on an LPL Knockout Background. To investigate the metabolic consequences of tissue-specific expression of LPL in the heart, transgenic mouse lines were generated that expressed the enzyme in CM but not in SM or AT. A breeding strategy with two crosses was necessary to produce littermates that were either controls (L2) or that expressed hLPL in CM on either the wild-type (L2-hLPL) or the knockout (L0-hLPL) background (Table 1). LPL gene expression was assessed by mRNA concentrations in CM, SM, and AT. Mouse-specific LPL mRNA (3.8 kb) (Fig. 1*D*) was detected in Northern blotting experiments in all of these tissues from L2 and L2-hLPL mice. In contrast, no signal was observed in any of these tissues from L0-hLPL animals. When a hLPL-specific probe was used, a signal of 3.6 kb (Fig. 1*D*) was found in CM of L2-hLPL and L0-hLPL mice. No hLPL mRNA was detected in AT or SM of the examined mouse lines. Expectedly, no hLPL mRNA signal was found in any tissue of L2 control mice. In summary, L2-hLPL animals express mouse LPL in a normal tissue-specific pattern and additionally express hLPL mainly in CM. L0-hLPL mice lack the endogenous mouse LPL gene but still express the hLPL transgene in CM. Thus, L0-hLPL mice lack LPL in SM and AT. L0-hLPL mice survive the lethal hypertriglyceridemia observed in LPL knockout mice within 24 h after birth. They grow normally over the first 2 months and exhibit an identical body-mass composition compared with controls and L2-hLPL animals.

Table 1. Summary of genotypes

Genotype	Mouse LPL alleles	Trangene alleles	Description
L2	2	0	Control mice
L2-hLPL	2	1	Express the transgene in addition to the endogenous LPL alleles
L0-hLPL	0	1	Express the transgene but lack functional mouse LPL alleles

Lipolytic Enzymes. To confirm the tissue-specific expression pattern on the level of protein expression, LPL-activity determinations were performed with tissue extracts from CM, SM, and AT. Additionally, LPL activities were measured in PHP (Table 2). In fasted and fed L2-hLPL mice, LPL activity in CM was increased by 130% and 20%, respectively, whereas it remained normal in AT and SM compared with L2 mice. LPL activity in CM of fasted and fed L0-hLPL mice was decreased 34% and 41%, respectively, whereas it was undetectable in SM and AT. In the fasted state, PHP-LPL activities were increased by 10% in L2-hLPL mice and decreased 67% in L0-hLPL mice compared with L2 mice. PHP-LPL activities of fed mice were increased by 180% in L2-hLPL mice and decreased by 18% in L0-hLPL mice. In both fed and fasted mice, hepatic TG lipase activities were normal in L2-hLPL mice but slightly decreased in L0-hLPL mice.

Plasma TG Metabolism. The effects of CM-specific expression of LPL on the plasma lipid profile in both fed and fasted animals are summarized in Table 3. Relative to control mice, TG levels of L2-hLPL and L0-hLPL mice decreased 60% and 18%, respectively, in the fed state. In the fasted state, L2-hLPL mice showed a 50% reduction, whereas L0-hLPL mice exhibited a 28% reduction in TG plasma levels. To study the metabolic cause of the reduced TG seen in both L2-hLPL and L0-hLPL mice, VLDL-turnover experiments were performed; [^{3}H]VLDL, metabolically labeled via injection of [^{3}H]palmitate into control animals, was injected in animals, and the disappearance of radioactivity was followed over 40 min. As shown in Fig. 2, radioactive VLDL disappeared more quickly from L2-hLPL and L0-hLPL mouse plasma than from control plasma within the first 20 min of the

Table 2. Fed and fasted lipolytic activities in tissues and PHP

		LPL activity in tissue, μmol of FFA per g per h			Lipolytic activity in PHP, μmol of FFA per ml per h	
Genotype	*n*	CM	SM	AT	LPL	Hepatic TG lipase
Fasted						
L2	5	17.8 ± 5.6	1.55 ± 0.22	4.85 ± 4.17	10.1 ± 5.0	2.79 ± 0.82
L2-hLPL	5	40.5 ± 5.0**	1.51 ± 0.38	3.98 ± 2.97	11.1 ± 6.2	2.56 ± 0.89
L0-hLPL	5	11.7 ± 6.2	0.26 ± 0.23**	0.27 ± 0.26*	3.4 ± 1.4**	1.54 ± 0.51
Fed						
L2	7	19.0 ± 5.6	2.18 ± 0.77	8.22 ± 6.13	7.10 ± 2.84	2.46 ± 0.63
L2-hLPL	7	23.6 ± 6.6	1.73 ± 0.64	9.09 ± 7.35	12.90 ± 6.22*	2.75 ± 0.61
L0-hLPL	6	11.1 ± 3.3*	0.06 ± 0.05**	0.28 ± 0.18*	5.83 ± 1.43	1.79 ± 0.60

Animals of both sexes were used. All values represent means ± SD. n = number of animals.
*, $P < 0.05$ compared to L2; **, $P < 0.01$ compared to L2.

decay curve. The VLDL fractional catabolic rate in L2-hLPL and L0-hLPL mice was increased by 2.6-fold and 2.2-fold, respectively, compared with control littermates (Table 4). The VLDL production rate was not changed.

Plasma TG levels also were measured during the suckling period to find out whether CM-specific LPL expression was sufficient to catabolize the large fat load under this condition (Fig. 3). Animals of all genotypes were analyzed at the ages of 30 h, 1 week, 2 weeks, and 3 weeks. At all time points, plasma TG levels in L2-hLPL animals were generally 30–40% lower compared with L2 mice. In contrast, L0-hLPL animals exhibited a 10-fold increase in plasma TG levels 30 hours after birth. Subsequently, TG levels decreased and, at the time of weaning, reached levels that were lower than those in control mice.

Plasma Cholesterol and FFA Metabolism. L2-hLPL mice showed an increase in HDL-cholesterol concentrations in the fasted (8%) and the fed (16%) state. Fed L0-hLPL mice also showed a 10% increase in HDL-cholesterol levels compared with controls, whereas fasted animals exhibited no significant difference from controls. The small changes in HDL-cholesterol levels were not sufficient to increase significantly the total cholesterol level in the plasma of L2-hLPL and L0-hLPL animals. FFA concentrations also were similar across the various mouse lines.

Morphology, Histopathology, and Tissue Fatty Acid Composition. Morphological and histochemical analysis of CM, SM and AT in light and electron microscopy did not identify significant changes in any of these tissues in adult animals. However, SM of L0-hLPL animals exhibited consistently less periodic acid-Schiff staining than control animals, suggesting a low glycogen content. Although body-mass composition as well as SM and AT morphology were grossly normal in L2-hLPL and L0-hLPL mice, large differences were found with regard to "fat-quality" when the FFA composition of AT and SM was analyzed. The results are summarized in Table 5. In L2-hLPL animals, the effects were moderate. A trend toward decreased polyunsaturated fatty acid (PUFA) concentrations in SM and AT was apparent; however, the difference was not statistically significant. In agreement with the decrease in PUFA, a small but significant increase was observed in 16:1 fatty acids in both SM and AT. In L0-hLPL animals, the effects were much more pronounced. The relative amounts of 18:2 and 18:3 fatty acids in SM of L0-hLPL mice were decreased by 74% and 81%, respectively, compared with L2 animals. In AT, 18:2 and 18:3 fatty acids were decreased by 87% and 88%, respectively. This decrease was balanced by increases in fatty acids that can be synthesized endogenously, namely, 14:0 (+43% in SM; +123% in AT), 16:0 (+20% in SM; +27% in AT), 16:1 (+90% in SM; +300% in AT), and 18:1 (+38% in SM; +23% in AT). These marked changes in PUFA content in SM and AT of L0-hLPL animals indicated that these tissues are hampered severely in the import of essential fatty acids from plasma.

DISCUSSION

The generation of transgenic mouse lines with a hLPL minigene that was fused to 8 kb of 5′ regulatory sequences of the mouse LPL gene resulted in animals that expressed hLPL predominantly in CM but not in SM and AT (L2-hLPL mice). The kinetics and steady-state levels of hLPL mRNA and enzyme activity during mouse development match those of the endogenous mouse LPL and are in good agreement with published studies on the development of mouse CM LPL during the first weeks of life (17, 18). Breeding L2-hLPL mice with L1 mice resulted in animals with various genotypes, including L2 control mice, L2-hLPL transgenic mice, and L0-hLPL mice. L0-hLPL mice lacked both functional endogenous mouse LPL alleles but expressed the hLPL transgene in CM. As expected, these animals exhibited no detectable LPL activity in SM and AT. LPL expression in CM alone was sufficient to rescue LPL knockout mice from lethal postnatal hypertriglyceridemia, despite the fact that CM-LPL activity was subnormal. The animals survive the suckling period,

Table 3. Fed and fasted plasma lipids

Genotype	n_1	TG, mg/dl	Total cholesterol, mg/dl	HDL-cholesterol	n_2	FFA, mmol/L
Fasted						
L2	20	77 ± 27	80 ± 13	71 ± 9	12	0.96 ± 0.28
L2-hLPL	16	38 ± 10**	86 ± 12	77 ± 10*	12	0.92 ± 0.27
L0-hLPL	14	56 ± 18*	89 ± 17	82 ± 13*	8	0.76 ± 0.19
Fed						
L2	28	96 ± 42	76 ± 15	62 ± 13	8	0.57 ± 0.21
L2-hLPL	26	39 ± 11**	83 ± 14	72 ± 15*	8	0.43 ± 0.12
L0-hLPL	22	79 ± 39	81 ± 20	68 ± 21	8	0.53 ± 0.24

Animals of both sexes were used. All values represent means ± SD. n_1 = number of animals used for TG, total cholesterol and HDL-cholesterol determination. n_2 = number of animals used for FFA determinations.
*, $P < 0.05$ compared to L2; **, $P < 0.01$ compared to L2.

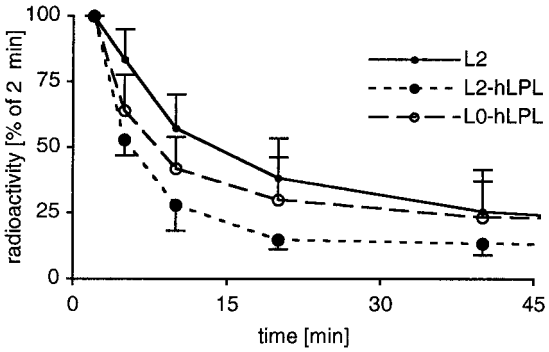

FIG. 2. Clearance of ^{3}H-labeled VLDL TG in male transgenic and control mice. The ^{3}H-labeled VLDL TG was administered to eight L2, five L2-hLPL, and six L0-hLPL mice, and serum radioactivity was determined at intervals over a period of 40 min. Data represent the percentage of radioactivity at 2 min after injection and are expressed as means ± SD.

develop normally to adulthood, and have a normal life span. The lack of LPL in AT and SM had no effect on body weight or body-mass composition.

The tissue-specific role of CM LPL was studied with regard to its metabolic effects on plasma lipoproteins. Overexpression of LPL in CM of L2-hLPL animals caused a moderate increase in PHP-LPL activity and was associated with decreased plasma TG and increased HDL-cholesterol concentrations. These results are consistent with the hypothesis that increased lipolysis of TG-rich lipoproteins supplies more precursor particles ("surface remnants") and, therefore, induces increased HDL biosynthesis. In contrast, the results obtained in L0-hLPL mice were unexpected. Subnormal LPL activity in CM (−34%) and in PHP (−60%) and the absence of LPL in AT and SM were associated with low plasma TGs and normal or slightly increased HDL-cholesterol levels. These findings showed that LPL expression in CM was sufficient to normalize the plasma TG metabolism in mice and suggested a potent role of CM LPL in the hydrolysis and clearance of TG-rich lipoproteins. Indeed, as determined in VLDL-turnover experiments, both L2-hLPL and, more unexpectedly, L0-hLPL animals had a significantly increased fractional catabolic rate, indicating an increased catabolism of VLDL particles compared with control mice. Because no association was observed between PHP LPL and plasma TG concentrations in our animals, we assume that PHP-LPL activity as a measure of "total-body LPL" is unsuited as a predictor of plasma TG levels. The current study indicates that CM LPL has this strong capacity for clearing TG-rich lipoproteins and is consistent with earlier studies with humans (7, 19) and mice (11, 12) reporting better negative correlations between muscle LPL and plasma TG levels than with the PHP-LPL activity.

In fed L0-hLPL animals, the decrease of plasma TG levels compared with L2 animals was less pronounced than in fasted animals. This result indicated a decreased tolerance against dietary fat. Therefore, plasma TGs were measured in animals of all genotypes on a very high-fat diet, namely, during the suckling period. Rodent milk contains an ≈3-fold higher fat content than human milk (20), and it can be estimated that 90% of nutritional calories originate from fat. Whereas L2-hLPL mice exhibited decreased TG levels in plasma during the whole suckling period, L0-hLPL animals developed a severe hypertriglyceridemia within the first hours after suckling started. Subsequently, the plasma TG values decreased gradually and reached normal values at the time of weaning. Apparently, mice that express LPL in CM, but not in AT and SM, are more fat-intolerant compared with control or L2-hLPL mice.

Table 4. Fractional catabolic rates and production rates

Genotype	*n*	TG mg/dl	Fractional catabolic rate, pools per h	Production rate, pools per h
L2	8	131 ± 18	5.7 ± 1.5	0.25 ± 0.08
L2-hLPL	5	55 ± 17*	15.0 ± 2.8*	0.27 ± 0.09
L0-hLPL	6	54 ± 20*	12.6 ± 2.7*	0.22 ± 0.08

Male animals were used. All values represent means ± SD. *n* = number of animals.
*, $P < 0.05$ compared to L2.

The fate of postlipolytic FFA has not been addressed in the current study. However, it is interesting to note that plasma FFA concentrations were similar in control animals and both transgenic mouse lines. This result supports the view that the FFAs that have been hydrolyzed by LPL in CM are mostly absorbed by cardiac tissue and not transported to other organs. Normal ketone body concentrations (not shown) and normal VLDL production rates in all mouse lines, as determined in VLDL-turnover experiments, provided additional evidence that increased FFA transport to the liver does not occur in L2-hLPL or L0-hLPL animals.

HDL-cholesterol levels were normal or slightly increased in L2-hLPL and L0-hLPL mice. This observation suggested a potent role specifically for CM LPL in HDL synthesis. Current evidence (21) indicates that LPL-mediated hydrolysis of TG-rich lipoproteins produces precursor surface remnants that become transformed to HDL particles by cholesterol-esterification and lipid-transport processes. The observation that PHP-LPL activities in our animals are not correlated with HDL-cholesterol concentrations suggests that, rather than total-body LPL, the tissue-specific expression of LPL in the heart at least partially determines plasma HDL concentrations. CM LPL hydrolyzes sufficient quantities of TG-rich lipoproteins to provide both low plasma TG levels and normal plasma HDL-cholesterol concentrations. These observations are in good agreement with clinical studies in humans where consistently better correlations were observed between muscle LPL and plasma TG and HDL-cholesterol concentrations than for PHP-LPL activity (22–24).

Transgene overexpression or exclusive expression did not cause major morphological changes in CM, indicating that the heart is able to cope with the increased rate of TG lipolysis. Similarly, in the absence of LPL in SM and AT, both tissues appeared to be normal in light- and electron-microscopic analyses. However, when the FFA composition of AT and SM from L0-hLPL mice was compared with the fatty acid composition of control animals, a drastic reduction in the content of PUFA was observed. Regarding the effect in AT, these findings confirm our earlier observations in mice that expressed LPL predominantly in SM (14). In an extension of these studies, we now show that LPL deficiency also results in a drastic decrease of PUFA in SM. The 75–88% decrease in linoleic and linolenic acid was counteracted by an increase in the saturated palmitic and stearic acid and their respective desaturase products, palmitoleic and oleic acid. These

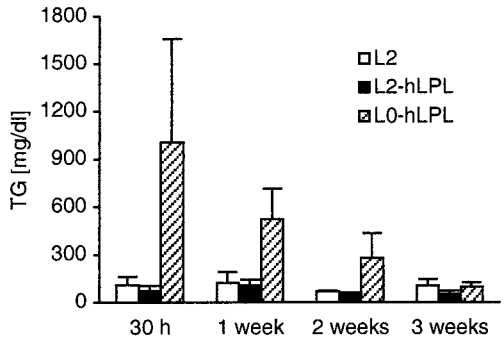

FIG. 3. Plasma TG levels during the suckling period. Plasma TG concentrations were determined in 5–10 animals of each genotype at the indicated time after birth. Bars represent mean values ± SD.

Table 5. Fatty acid composition in tissues

Genotype	*n*	Fatty acid			
		16:0	16:1	18:1	18:2 + 18:3
CM					
L2	6	22.9 ± 1.1	2.0 ± 0.6	15.7 ± 2.5	27.4 ± 2.5
L2-hLPL	5	21.7 ± 1.3	1.4 ± 1.1	12.2 ± 1.9	28.6 ± 1.5
L0-hLPL	6	23.1 ± 1.3	4.0 ± 2.3	18.7 ± 5.0	23.9 ± 2.8
SM					
L2	6	24.1 ± 2.3	6.8 ± 0.9	26.7 ± 3.8	33.7 ± 3.6
L2-hLPL	5	24.9 ± 1.1	8.3 ± 1.4*	27.0 ± 0.9	30.2 ± 2.8
L0-hLPL	6	29.1 ± 2.0*	12.8 ± 3.3*	36.6 ± 5.7*	8.7 ± 1.3**
AT					
L2	6	20.5 ± 1.5	6.4 ± 0.8	30.9 ± 1.4	38.8 ± 2.4
L2-hLPL	5	20.4 ± 1.3	9.4 ± 1.5*	29.0 ± 3.3	37.7 ± 3.4
L0-hLPL	6	26.0 ± 1.6**	25.8 ± 6.3**	38.1 ± 5.1*	5.0 ± 0.6**

Male animals were used. All values represent means ± SD. n = number of animals.
*, $P < 0.05$ compared to L2; **, $P < 0.001$ compared to L2.

results are consistent with the concept that LPL alone regulates the entry of TG-derived FFA in AT and SM. However, in the absence of the enzyme, these tissues do not become depleted in FFA but, alternatively, carry out *de novo* fatty acid synthesis, most likely from glucose. As a consequence, AT mass and body composition remain unchanged, whereas the composition of the lipid content in AT and SM changes markedly. The functional consequences of these changes on intracellular lipid metabolism, membrane function, arachidonic acid metabolism, etc. remain to be elucidated.

The pattern of tissue-specific LPL-transgene expression suggested that 8 kb of 5′ regulatory sequences of the mouse LPL gene contains the obligatory transcription elements for cardiac-specific LPL expression but lacks important AT and SM cis-regulatory elements. It is of interest that the regulatory regions responsible for LPL transcription in CM and SM apparently are segregated on the LPL promoter. This finding is consistent with earlier observations in transgenic mouse lines in which expression of the β-myosin heavy chain gene in CM also was controlled by a particular region that was distinct from the tissue-specific control elements in SM (25). The lack of transgene expression in AT of transgenic mice was unexpected, because earlier transfection/deletion analyses in cultivated adipogenic cell lines had defined a region close to the transcriptional start site as sufficient for adipocyte-specific LPL expression (26–28). The lack of LPL expression in AT in our animals might be explained by the presence of a strong transcriptional suppressor on the regulatory sequences of the transgene. Evidence for the existence of a negative transcriptional element far upstream in the regulatory region of the LPL gene was provided by Enerbäck *et al.* (27). The question as to whether the presence of LPL mRNA in kidney and aorta is caused by contaminating AT or actual transgene expression in these tissues remains to be determined.

Our results show that LPL expression in CM alone ensures normal animal development with regard to body weight and body composition. Additionally, moderate LPL expression in CM normalizes the plasma-lipoprotein pattern by providing for low plasma-TG and normal HDL-cholesterol levels even when the enzyme is absent in AT and SM. This result emphasizes the importance of an as-yet incompletely recognized tissue-specific role of CM LPL in the energy and lipoprotein metabolism of vertebrates.

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