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COOH-terminal Disruption of Lipoprotein Lipase in Mice Is Lethal in Homozygotes, but Heterozygotes Have Elevated Triglycerides and Impaired Enzyme Activity*

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The role of the enzyme lipoprotein lipase (LPL) in atherosclerosis is uncertain. To generate an animal model of LPL deficiency, we targeted the LPL gene in embryonic stem cells with a vector designed to disrupt the COOH terminus of the protein and used these cells to generate LPL-deficient mice. Germ line transmission of the disrupted LPL allele was achieved with two chimeric males, and offspring from each of these animals were phenotypically identical. Pups homozygous (−/−) for LPL deficiency died within 48 h of birth with extreme elevations of serum triglycerides (13,327 mg/dl) associated with essentially absent LPL enzyme activity in heart and carcass. Newborn heterozygous (+/−) LPL-deficient pups had lower LPL enzyme activity and higher triglycerides (370 *versus* 121 mg/dl) than wild type (+/+) littermates. Adult heterozygotes had higher triglycerides than wild type mice with *ad libitum* feeding (236 mg/dl for +/− *versus* 88 mg/dl for +/+) and after fasting for 4 h (98 mg/dl for +/− *versus* 51 for +/+) or 12 h (109 mg/dl for +/− *versus* 56 mg/dl for +/+). Triglycerides were present as very low density lipoprotein particles and chylomicrons, but high density lipoprotein cholesterol levels were not decreased in +/− animals. Plasma heparin-releasable LPL activity was 43% lower in +/− *versus* +/+ adult animals. LPL activity, mRNA, and protein were lower in the tissues of +/− *versus* +/+ mice.

Homozygous LPL deficiency caused by disruption of the COOH terminus of the enzyme is lethal in mice. Heterozygous LPL deficiency caused by this mutation is associated with mild to moderate hypertriglyceridemia without affecting static HDL cholesterol levels. Heterozygous LPL-deficient mice could be useful for determining if hypertriglyceridemia, independently or in combination with other discrete defects, influences atherosclerosis.

Hypertriglyceridemia is a risk factor for coronary heart disease (1), but the biology underlying this epidemiology is obscure. Elevated triglycerides may cause vascular damage through both direct and indirect mechanisms. Remnants of triglyceride-rich lipoproteins may be directly atherogenic (2), a hypothesis bolstered by the recent demonstration of triglyceride-rich lipoproteins in human atherosclerotic lesions (3). Elevated triglycerides might affect vascular health indirectly by decreasing HDL[1] cholesterol levels, by rendering LDL particles more atherogenic, or by affecting clotting.

Defects in lipoprotein lipase (LPL) can cause hypertriglyceridemia. LPL is increasingly recognized as a multifunctional protein. In addition to hydrolyzing triglycerides in VLDL and chylomicrons, the LPL protein may also function as an apolipoprotein, associating with the surface of various lipoproteins to promote binding to the low density lipoprotein (LDL) receptor-related protein/α_2-macroglobulin receptor (4), LDL receptor (5, 6), and extracellular proteoglycans (7, 8). The LPL protein can be functionally divided into two major domains: an NH_2-terminal domain containing the catalytically active site, and a COOH-terminal domain (9). The latter is essential for catalytic activity (10), binds lipoproteins (11), and is probably responsible for the LPL-mediated catabolism of triglyceride-rich lipoproteins by the LDL receptor-related protein/α_2-macroglobulin receptor (12).

LPL could also impact atherogenesis independent of effects on circulating triglycerides. Macrophages from human and rabbit atherosclerotic lesions express LPL (13–15), and higher levels of macrophage LPL expression are found in inbred strains of mice that are susceptible to atherosclerosis (16), suggesting that local expression of LPL could promote uptake of lipoproteins by vascular tissue (17). Thus genetic defects in LPL could have opposing effects on atherosclerotic risk; systemically decreased function could increase the concentration of atherogenic, triglyceride-rich particles, but decreased macrophage expression could decrease foam cell formation.

Human heterozygous LPL deficiency is probably common. It has been postulated to form a subset of familial combined hyperlipidemia (18), a very common genetic disorder associated with vascular disease. However, heterozygous LPL deficiency could have phenotypically different effects depending on genetic and physiological backgrounds. This might explain why some families with heterozygous LPL deficiency manifest familial hypertriglyceridemia (19), while others have lipid abnormalities associated with increased atherosclerotic risk (20). Further complicating study of human heterozygous LPL defi-

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[1] The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; kb, kilobase pair(s); bp, base pair(s); PCR, polymerase chain reaction; apo, apolipoprotein.

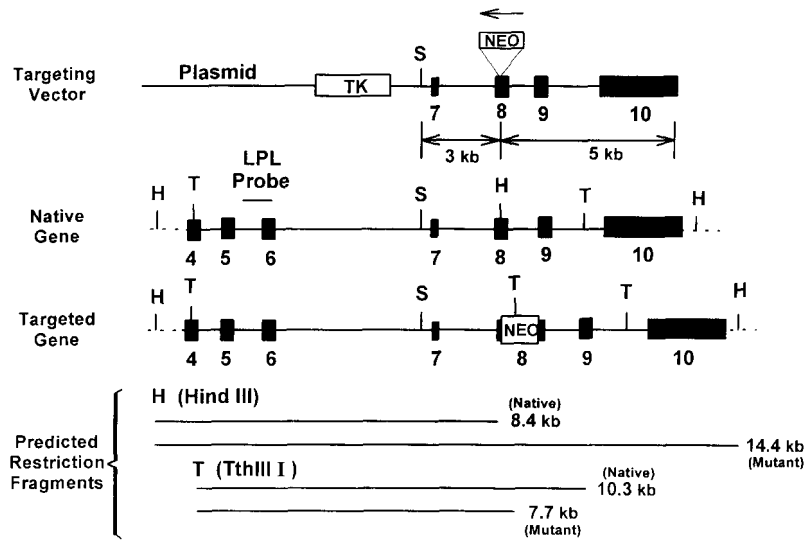

FIG. 1. **Strategy for inactivation of the mouse LPL gene.** Schematic diagrams of the targeting vector, native (wild type) mouse gene, a successfully targeted allele, and the predicted restriction fragments resulting from digestion of genomic DNA with *Hin*dIII and *Tth*111I are shown. *S* represents *Sac*I, *TK* represents thymidine kinase (for negative selection in the presence of ganciclovir), and *NEO* represents the neomycin resistance cassette (for positive selection with G418). Also shown is the location of the LPL genomic probe (spanning the intron 5/exon 6 junction) used for detection of fragments by Southern blotting.

ciency is the fact that detection of this disorder is difficult given the large number of mutations in the LPL gene (21).

With a goal of generating an animal model of LPL deficiency within a homogeneous genetic background suitable for studying atherosclerosis, we have inactivated the LPL gene in mice by homologous recombination using a targeting vector designed to disrupt the COOH-terminal domain of the LPL protein.

EXPERIMENTAL PROCEDURES

Vector Construction and Generation of LPL-deficient Mice—An 8.3-kb *Sac*I/*Sau*3AI genomic fragment was isolated from a λEMBL3 library constructed using Balb/c DNA (22). Restriction mapping, Southern blotting (with detection by oligonucleotides based on the mouse LPL cDNA sequence in Ref. 23), and sequencing showed that this fragment contained exons 7–10 of the mouse gene with essentially the same structure described by Zechner *et al.* (24). A 1.8-kb *Eco*RI/*Hin*dIII fragment of the plasmid pKJ-1 containing the neomycin resistance cassette driven by the PGK promoter was inserted by blunt-end ligation at a *Hin*dIII site in exon 8. This manipulation was shown to abolish the *Hin*dIII site and interrupt exon 8, resulting in a predicted mouse LPL protein disrupted following the leucine residue at position 380 as numbered in Ref. 23. This modified genomic clone was then inserted at the *Xho*I site of a Bluescript plasmid (a gift from Fred Fiedorek, Chapel Hill, NC) containing the 3.4-kb thymidine kinase fragment from pHSV-106 to generate the targeting vector.

E14 ES cell electroporation, positive (using G418) and negative (using ganciclovir) selection, and injections into the blastocysts of C57BL/6J embryos were carried out as described (25). Chimeric (on the basis of coat color) males were mated with C57BL/6J females to generate F1 animals, and these F1 animals were crossed with each other to generate the mice characterized in this study.

Animal Genotyping—DNA isolated from tail (for most animals) or liver (from recently expired animals) was subjected to Southern blotting or PCR. For Southern blotting, 5–15 μg of DNA was electrophoresed followed by acid depurination, treatment with NaOH, and transfer using standard techniques. Blots were probed with a random-primed 0.8-kb *Bam*HI/*Pvu*II fragment of the mouse LPL gene spanning the 3′ end of intron 5 and the 5′ end of exon 6. As expected, preliminary blots showed that this probe did not hybridize with the targeting vector. For PCR genotyping of potential heterozygotes, an upstream primer corresponding to mouse exon 8 (5′-TTT ACA CGG AGG TGG ACA TCG GA) and a downstream primer corresponding to a region near the 3′ end of the neomycin resistance cassette (5′-TCG CCT TCT ATC GCC TTC TTG AC) were used in reactions containing genomic DNA and 2 mM $MgCl_2$ subjected to the following cycling parameters: 5 min at 94 °C for 1 cycle, and 1 min at 94 °C/2 min at 55 °C/3 min at 72 °C for 30 cycles.

Mouse Housing, Handling, and Diet—Animal rooms were illuminated between 7:00 a.m. and 7:00 p.m. Mice were fed a 50/50 mixture of PicoLab rodent chow 20 and mouse chow 20 (product numbers 5053 and 5058) with a total fat content of ~6.75%. Animals were weaned at 21 days of age. For blood collection via the retro-orbital plexus, animals were lightly anesthetized with methoxyflurane. Deep anesthesia was used for those animals subjected to exsanguination via inferior vena cava venipuncture.

Lipid and Lipoprotein Analysis—Triglycerides, cholesterol, and phospholipids were assayed enzymatically using commercially available kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). For measurements of total lipids, serum was kept at 4 °C or on ice and assayed within 2 h for triglycerides and phospholipids to decrease the chances of endogenous hydrolysis (26). Cholesterol determinations were generally done the following day after storing samples at −70 °C. HDL cholesterol was measured after polyethylene glycol precipitation of apo B-containing lipoproteins (27). For separation of lipoproteins by gel filtration, serum samples from two animals with similar total lipids were pooled and separated by FPLC using two Superose 6 columns in series (28). The 50 fractions collected per condition were analyzed the same day for triglycerides and the following day for phospholipids and cholesterol.

Determination of LPL Enzyme Activity, mRNA, and Protein—LPL activity was assayed as the salt-inhibitable ability of triplicate samples to hydrolyze an emulsion containing radiolabeled triolein (29). For postheparin plasma activity, animals were injected intraperitoneally with 200 units of porcine heparin; 30 min later blood was collected from the inferior vena cava. Previous studies have shown this procedure to be suitable in mice (30). For other assays, individual tissues were flash frozen in liquid nitrogen, weighed, and made 4% (w/v) in assay buffer (29). For newborn pups, the heart was removed and processed, the milk-filled stomach was removed and discarded (to prevent dilution of radioactive emulsion by milk lipids), and the remaining carcass (minus heart and stomach) was frozen, weighed, and homogenized in assay buffer. For determination of LPL message, total RNA was prepared from tissues by centrifugation in cesium chloride and mRNA was detected by Northern blotting as described previously (31). For determination of LPL protein, tissue extracts were subjected to SDS-polyacrylamide gel electrophoresis and Western blotted, and LPL protein was detected with chicken anti-bovine milk LPL as described (29). Each gel included one lane loaded with purified bovine LPL as a positive control.

Statistical Information—Differences were assessed using unpaired, two-tailed *t* tests unless otherwise specified. For the data shown in Fig. 4, ANOVA was specifically not used because groups contained different numbers of animals.

RESULTS

The strategy for inactivating the mouse LPL gene is shown in Fig. 1. The mouse LPL gene has 10 exons, 9 of which are translated. The targeting vector contains ~3 kb of mouse DNA upstream and ~5 kb downstream of the exon 8 *Hin*dIII site where the neomycin resistance cassette (NEO) was inserted. Predicted restriction fragments detected by the LPL probe spanning the intron 5/exon 6 junction are shown at the bottom of the figure for native and targeted genes. With *Hin*dIII, the native fragment is 8.4 kb but the targeted fragment is 14.4 kb since insertion of NEO abolishes the natural *Hin*dIII site in

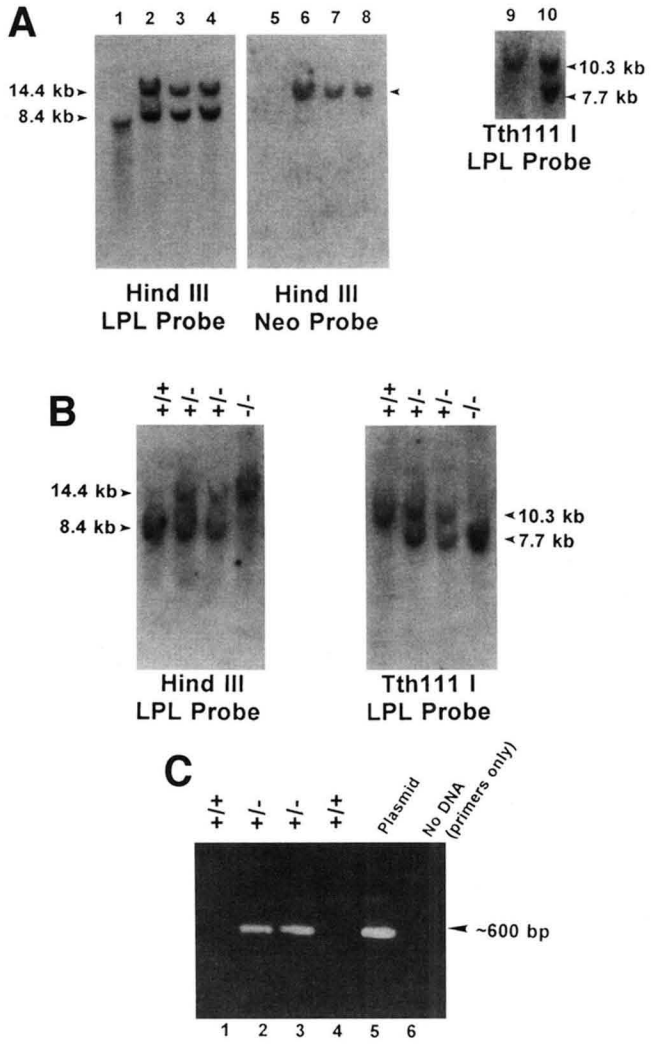

FIG. 2. **Southern blots and PCR assays of correctly targeted ES cells and mice.** *Panel A* shows representative Southern blots of ES cells. Fifteen μg of genomic DNA from non-electroporated ES cells (*lanes 1*, *5*, and *9*) and correctly targeted ES cell clones (*lanes 2-4*, *6–8*, and *10*) was digested with *Hin*dIII or *Tth*111I, Southern blotted, and detected with the LPL probe indicated schematically in Fig. 1 (*lanes 1–4*, *9*, and *10*) or a NEO probe (*lanes 5–8*), consisting of a *Bam*HI/*Pvu*II fragment complementary to the phosphotransferase coding region from the vector pKJ-1. Correctly targeted *Hin*dIII-cut DNA contained the predicted 14.4-kb mutant band, which hybridized with both LPL and NEO probes. Correctly targeted *Tth*111I-cut DNA contained the predicted 7.7-kb mutant band (*lane 10*). *Panel B* shows DNA from wild type (+/+), heterozygous (+/−), and homozygous (−/−) mice after *Hin*dIII or *Tth*111I digestion. As expected, heterozygotes have both mutant and native alleles after digestion with both enzymes while homozygotes have only the mutant allele (14.4 kb for *Hin*dIII, 7.7 kb for *Tth*111I). *Panel C* shows a PCR assay using the same DNA from the +/− mice in *panel B*. Assays were performed as described under "Experimental Procedures" using an upstream primer complementary to exon 8 of the mouse LPL gene and a downstream primer complementary to NEO. As expected, the predicted ~600-bp band was seen in DNA from +/− mice and with the targeting vector (labeled *Plasmid*) but not in +/+ mice or in the negative control lane (labeled *No DNA*).

exon 8. With *Tth*111I, the native fragment is 10.3 kb but the targeted fragment is 7.7 kb since NEO introduces a new *Tth*111I I site 5′ to the natural *Tth*111I site.

Evidence for correct targeting of the LPL gene in ES cells and mice is shown in Fig. 2. Southern blots of ES cell DNA are shown in *panel A*, blots of F1 × F1 mouse DNA are in *panel B*, and results of a PCR assay using mouse tail DNA are in *panel C*. For *panel A*, *lanes 1–4* contain *Hin*dIII-digested DNA detected with the LPL probe shown in Fig. 1. Wild type (+/+) cells contain only the 8.4-kb native fragment (*lane 1*), but targeted clones also contain the 14.4-kb targeted allele (*lanes 2–4*). When this same DNA was probed with a NEO cDNA (*lanes 5–8*), only the 14.4-kb targeted allele was detected in targeted clones (*lanes 6–8*), consistent with the presence of the NEO cassette in this fragment. *Tth*111I-digested DNA from +/+ cells showed only the 10.3-kb native band (*lane 9*), but the expected 7.7-kb band is also present in targeted cells (*lane 10*).

TABLE I
Neonatal mortality rates and serum lipids for LPL-deficient mice

	−/−	+/−	+/+
Spontaneous deaths[a]	6	4	2
Total born[a]	6	16	11
Mortality[a]	100%[b]	25%	18%
Triglycerides[c]	13,327 ± 1,868	370 ± 96	121 ± 12
Cholesterol[c]	834 ± 369	74 ± 3	61 ± 2
HDL cholesterol[c]	0	49 ± 9	30 ± 3
Phospholipids[c]	3,873 ± 969	145 ± 13	105 ± 7

[a] Data are from the progeny of the first three F1 × F1 crosses in St. Louis.
[b] $p = 0.0028$ *versus* +/− by Fisher's exact test.
[c] Data are presented as mean ± S.E. in mg/dl. For triglycerides, cholesterol, and phospholipids, $n = 3$ for −/−, $n = 12$ for +/−, and $n = 21$ for +/+. For HDL cholesterol, $n = 3$ for −/−, $n = 6$ for +/−, $n = 6$ for +/+.

Five male chimeric mice were generated using these ES cells. Two independent chimeras transmitted the mutant allele through the germ line, and analyses of progeny from each of these animals were identical. The same alleles detected in ES cells were detected in the offspring of F1 × F1 crosses (*panel B*). For *Hin*dIII, heterozygotes (+/−) carried both the 8.4-kb native and 14.4-kb mutant alleles while homozygotes (−/−) had only the 14.4-kb allele. For *Tth*111I, +/− animals carried both the 10.3-kb native and 7.7-kb mutant alleles while −/− mice had only the 7.7-kb fragment.

Additional confirmation of authentic targeting is shown in *panel C*. Tail DNA from animals shown to be +/+ or +/− by Southern blotting was subjected to PCR using an upstream primer just 5′ to the NEO insertion site in exon 8 and a downstream primer complementary to NEO. The expected ~600-bp band was amplified from heterozygotes (+/−, *lanes 2* and *3*) but not wild type animals (+/+, *lanes 1* and *4*). This assay was subsequently used for rapid genotyping. In other studies, confirmation of the homozygous state was also performed by PCR using the primers of *panel C* in conjunction with primers (separated by ~127 bp) flanking the NEO insertion site in exon 8. With the latter primers, wild type animals showed a 127-bp band, homozygotes had a ~1.8-kb band (due to the presence of the NEO cassette between the primers), and heterozygotes had both bands (data not shown).

Homozygotes were born viable, initially appeared healthy, but uniformly died within 48 h. The 100% mortality for this genotype was significantly higher than death rates for +/− and +/+ animals (Table I). Autopsy findings from four homozygotes (data not shown) were nonspecific, although atelectasis and congestion in the lungs and hepatic congestion were seen consistently. There were no pancreatic abnormalities detected.

Also shown in Table I are serum lipids from mice ~12 h after birth. Homozygotes had marked elevations of triglycerides, phospholipids, and total cholesterol, but no detectable HDL cholesterol. This extreme hyperlipidemia was feeding-dependent. One homozygote was kept from nursing (verified by the absence of milk in the stomach), and in this single animal at the time of sacrifice lipids were: triglycerides, 296 mg/dl; cholesterol, 95 mg/dl; phospholipids, 178 mg/dl. Triglycerides ($p = 0.0019$), cholesterol ($p = 0.0008$), and phospholipids ($p = 0.0057$) were significantly higher in heterozygotes than in wild

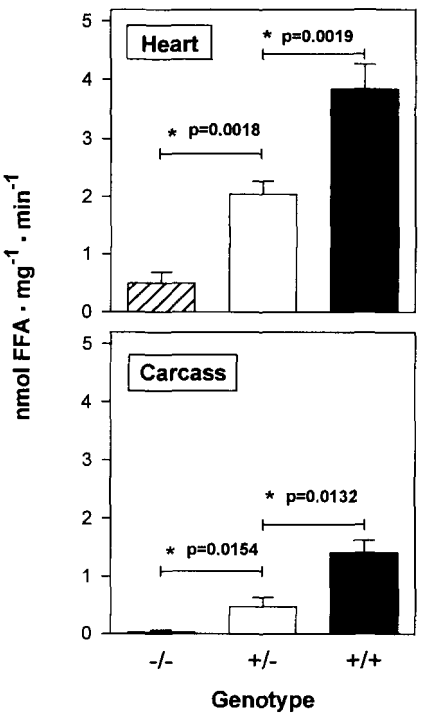

FIG. 3. **LPL enzyme activity in the heart and carcass of neonatal mice.** Pups were allowed to suckle and then sacrificed 12 h after birth. Tissues (not including the milk-filled stomach) were flash-frozen in liquid nitrogen and then thawed once for determination of LPL enzyme activity as described under "Experimental Procedures." Data represent means ± S.E. for 4 animals for −/−, 8 animals for +/−, and 6 animals for +/+, with assays for each animal done in triplicate.

type animals at 12 h. HDL cholesterol levels were not decreased in +/− animals; in fact, HDL cholesterol was almost significantly higher in +/− compared to +/+ pups ($p = 0.0730$).

As expected, LPL enzyme activity was decreased in +/− and essentially absent in −/− pups (Fig. 3). For these experiments, the heart was removed and assayed, then the carcass minus the heart (and milk-filled stomach, as described under "Experimental Procedures") was assayed separately.

Heterozygotes developed normally. Over 100 animals were weighed at weekly intervals for the first two months of life, and although +/− animals tended to be slightly heavier than +/+ littermates, this difference was not significant ($p = 0.5513$). Heterozygotes had higher triglycerides than wild type animals with fasting and with *ad libitum* feeding (Fig. 4 and Table II). With fasting, triglycerides were ~2-fold elevated in +/− *versus* +/+ animals regardless of sex. With *ad libitum* feeding, the genotype-specific differences in triglycerides were amplified with the highest elevations seen in heterozygous LPL-deficient females. There were no significant differences between groups for total cholesterol or phospholipids.

The fasting data of Fig. 4 were obtained after a 12-h fast (11:00 p.m. to 11:00 a.m.), an intervention that may represent a considerable physiological stress in mice (32). However, a 4-h fast (7:00 a.m. to 11:00 a.m.) resulted in essentially the same difference in triglycerides between +/− and +/+ animals (Table II).

As with neonates, HDL cholesterol levels were not lower in adult +/− animals. *Ad libitum* fed adults with heterozygous LPL deficiency tended to have higher HDL cholesterols than their +/+ littermates (118 ± 14 for +/− *versus* 100 ± 9 for +/+, $p = 0.3339$).

Lipoprotein analysis by gel filtration chromatography showed that the increased triglycerides in heterozygous LPL-deficient animals were due to an increase in VLDL/chylomicrons (Fig. 5, *top panels, fractions 1–10*). Consistent with measurements of HDL cholesterol by PEG precipitation, HDL cholesterol measured by gel filtration tended to be higher in +/− animals (*middle panels, fractions 30–38*). In males, "shoulders" to the right of the VLDL triglyceride peak (*fractions 10–15*) and to the left of the HDL cholesterol peak (*fractions 20–30*) were consistently more prominent in heterozygotes.

LPL enzyme activity was decreased in animals carrying one copy of the mutant allele (Fig. 6). Activity in plasma 30 min after the intraperitoneal injection of heparin (*panel A*) was 43% lower in heterozygotes (146 ± 25 μmol of FFA/ml/h for +/− *versus* 258 ± 19 for +/+, $n = 4$ animals per genotype, $p = 0.0121$). This decrease was also reflected in enzyme activity assayed in individual tissues (*panel B*). For heart, kidney, epididymal/parametrial and inguinal adipose tissue, lung, psoas muscle, and brain, LPL activity was consistently decreased in +/− *versus* +/+ animals. These differences were statistically significant only for heart and kidney (both $p < 0.05$) due to considerable animal-to-animal variation, especially for adipose tissue. Since these experiments were performed using the F2 generation representing a mixture of the the 129/Ola and C57BL/6J strains, the genetic heterogeneity of these animals is probably a major source of the observed variation in LPL enzyme activity.

There were tissue-specific differences in the magnitude of the decrease in enzyme activity associated with the mutant allele. For example, in multiple assays involving six different side-by-side comparisons of age and weight-matched +/+ *versus* +/− animals, the decrease in heart activity was consistently less than 50% (mean decrease 28.4%) while the decrease in renal activity was consistently greater than 50% (mean decrease 62.4%).

LPL message and protein were decreased in heterozygotes (Fig. 7). Both the 3.6- and 3.4-kb LPL mRNA species detected by others in mouse tissues (23) were decreased in +/− (*lane 2*) compared to +/+ (*lane 1*) adipose tissue. Similar differences were seen in comparisons of adipose tissue total RNA from five different pairs of +/+ and +/− animals. A decrease in LPL mRNA for heterozygotes was also seen in heart (not shown). LPL protein mass was decreased in +/− (*lane 4*) compared to +/+ (*lane 3*) adipose tissue. Similar differences were seen in comparisons of adipose tissue protein from three different pairs of +/+ and +/− animals.

DISCUSSION

We present evidence consistent with the inactivation of the LPL gene in mice. Targeting of the LPL gene with a vector designed to disrupt the COOH terminus of the LPL protein results in severe hypertriglyceridemia and essentially absent enzyme activity in homozygotes. These animals die within 2 days of birth. Heterozygotes have decreased enzyme activity and mild to moderate hypertriglyceridemia with both feeding and fasting. The homozygous and heterozygous states appear to have different effects on HDL levels.

Homozygous LPL deficiency in humans causes a distinctive phenotype, the chylomicronemia syndrome, that is not necessarily lethal (33). Naturally occurring defects in LPL activity have also been described in cats (34), mice lacking mast cells (W/W^v mice; Ref. 35), and mice homozygous for combined lipase deficiency (*cld/cld* mice; Ref. 36). Our homozygotes resemble *cld/cld* mice. Triglycerides are similar at 12 h postpartum (~10,000 mg/dl for *cld/cld*, ~13,000 mg/dl for LPL −/−), and animals uniformly die within 48 h of birth. *cld/cld* mice presumably die because of microinfarcts in critical organs caused by dense packing of chylomicrons in capillaries (37), and a similar mechanism probably operates in LPL −/− mice. The

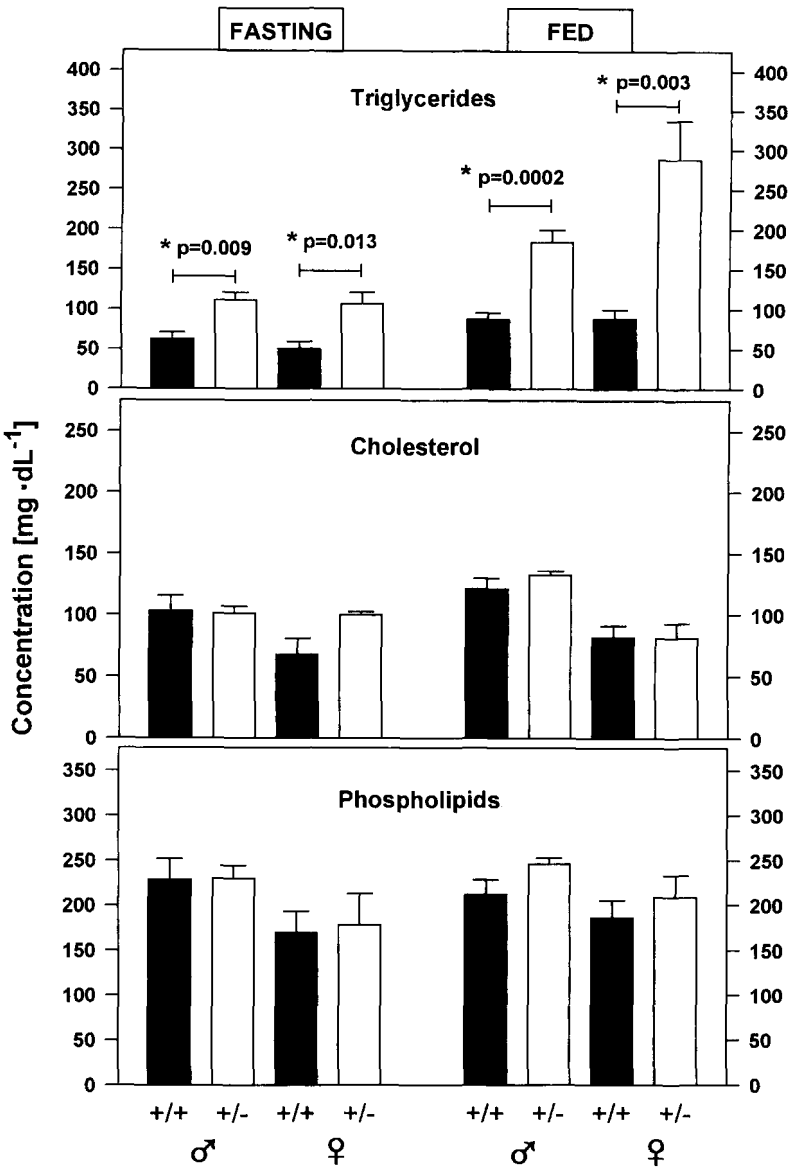

FIG. 4. **Serum lipids in adult mice.** Blood was obtained by retro-orbital bleeding from mice at age 7–9 weeks between 10:00 a.m. and noon. Serum was assayed immediately or stored at −70 °C for assay within 24 h. Data from the *left side* of the figure represent mice fasted for 12 h, and data from the *right side* represent *ad libitum* fed mice. +/+ indicates wild type, and +/− indicates heterozygotes as determined by PCR genotyping of tail DNA. Data represent means ± S.E. for 3–8 animals per group.

cld mutation affects the posttranslational processing of both LPL and hepatic lipase (38), but the similarities between LPL −/− and *cld/cld* mice suggest that LPL is the lipase most critical for neonatal survival.

HDL cholesterol was absent from the serum of LPL −/− mice, an expected finding since the major sources of lipid for HDL generation are thought to be remnant cholesterol and phospholipid from chylomicron/VLDL metabolism. Whether apolipoprotein A-I, the major protein of HDL, is produced normally in LPL −/− animals is unknown. These mice could be used to determine if nascent HDL-like particles are present in the absence of LPL activity.

In human populations, lower levels of LPL enzyme activity are associated with lower levels of HDL cholesterol (39). Given this association, one might predict that the lower (but not absent) LPL activity of LPL +/− mice would also be associated with lower HDL cholesterol. This was not observed. There was no difference in HDL cholesterol between +/− and +/+ adult mice, and levels were almost significantly higher in heterozygous neonates (Table I). At least three groups have overexpressed human LPL in transgenic mice (40–42), and all report decreases in triglyceride-rich lipoproteins without significant effects on HDL cholesterol. These results and our findings in LPL +/− mice suggest that, with the exception of the LPL −/− state, LPL activity in mice has a limited role in determining HDL cholesterol levels.

TABLE II
Serum triglycerides in adult heterozygous LPL-deficient and wild type mice

Data are presented as mean ± S.E. in mg/dl.

	+/−	+/+	*p*
Ad libitum feeding	236 ± 29	88 ± 7	0.0001
12-h fast	109 ± 8	56 ± 6	0.0001
4-h fast	95 ± 8	51 ± 5	0.0001

That heterozygous LPL-deficient mice have increased triglycerides seems sensible, but this result was not predictable. In humans, 94% of young heterozygotes have normal triglycerides (19) as do mice heterozygous for the *cld* mutation (36). The mechanism responsible for hypertriglyceridemia in our mice, especially with fasting, is uncertain. Mutations generally cause gain of function, loss of function, or have dominant negative effects. The latter class of mutation could be relevant to our mice since active LPL is probably a dimer and one could envision an inactive but stable monomer having a prolonged dominant negative effect on triglyceride metabolism. This mecha-

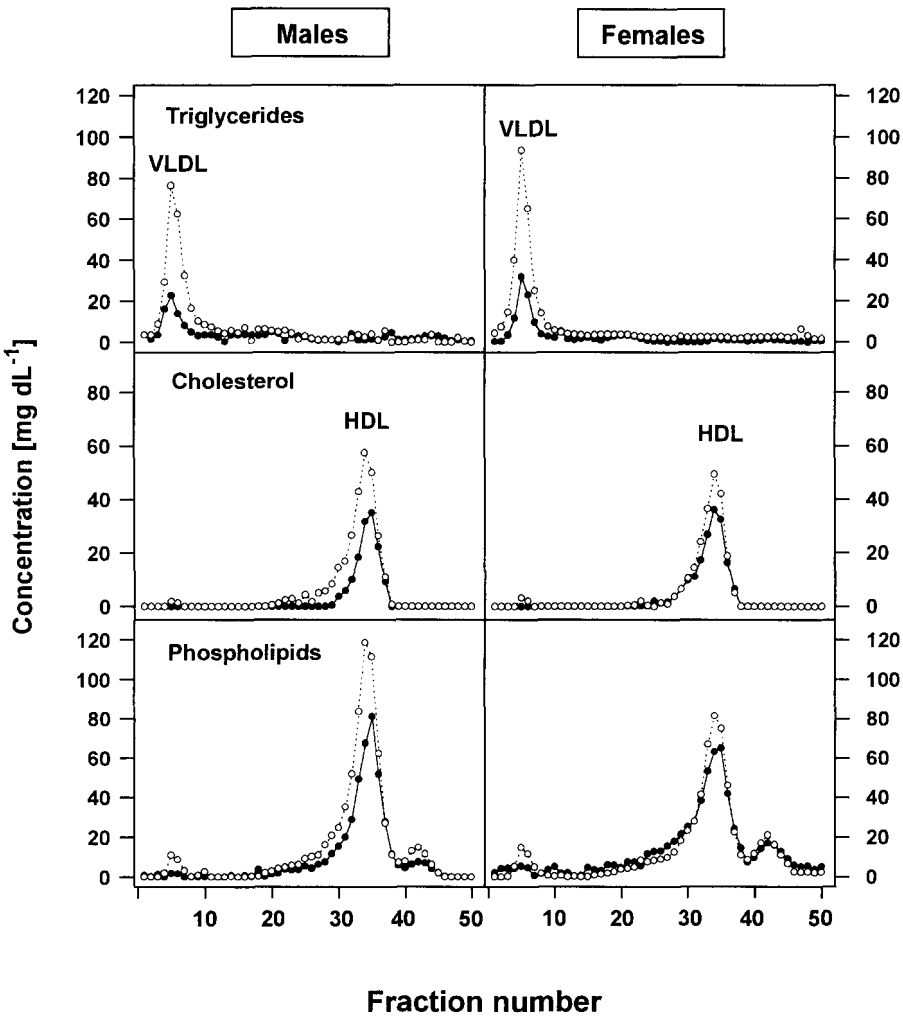

FIG. 5. **Separation of serum lipoproteins in mice by gel filtration chromatography.** Blood was obtained from the inferior vena cava under deep anesthesia as described under "Experimental Procedures." For each profile, serum from two *ad libitum* fed animals known to have similar lipid levels was pooled and separated using two Superose 6 columns in series. Fifty fractions were collected, and each was assayed for triglyceride, cholesterol, and phospholipid content. VLDL (which refers to both VLDL and chylomicrons since animals were studied in the fed state) and HDL peaks are appropriately labeled. The expected elution position for LDL is approximately fractions 20–25. Similar results were seen in at least two independent profiles for each sex and genotype. ●, wild type; ○, heterozygote.

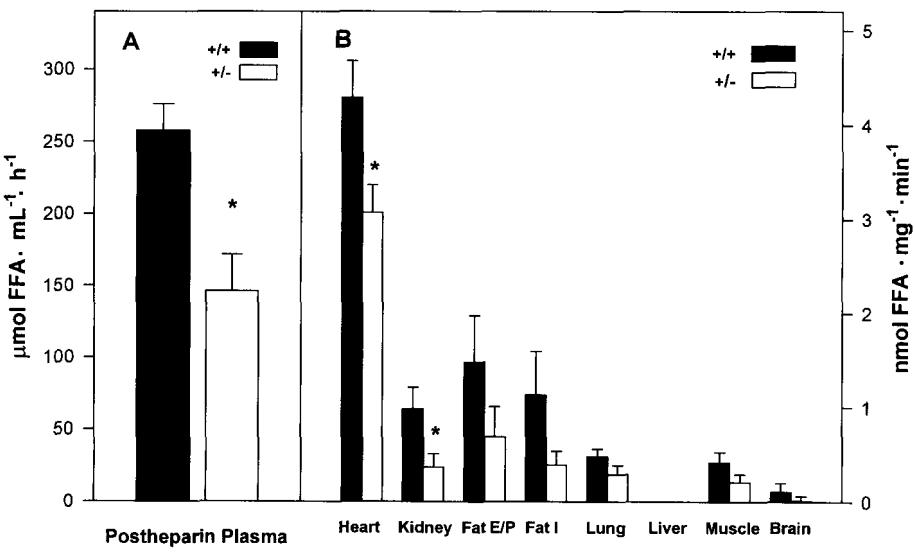

FIG. 6. **LPL enzyme activity in wild type (+/+, *solid bars*) and heterozygous LPL-deficient (+/−, *open bars*) mice.** For *panel A*, *ad libitum* fed animals were injected intraperitoneally with heparin and 30 min later plasma was obtained and LPL enzyme activity assayed as described under "Experimental Procedures." In *panel A*, * indicates $p = 0.0121$ *versus* +/+; data represent the mean ± S.E. for 4 animals (2 males and 2 females) for each genotype with plasma values for each individual animal determined in triplicate. For *panel B*, *ad libitum* fed animals (different from the animals used in *panel A*) were sacrificed and tissues flash-frozen in liquid nitrogen. *fat E* represents epididymal or parametrial adipose tissue, while *fat I* represents inguinal adipose tissue. In *panel B*, * indicates $p < 0.05$; data represent the mean ± S.E. for 6 animals (4 males and 2 females) for each genotype with each individual tissue value determined in triplicate. Sex differences were minimal except in fat, where epididymal/parametrial activities were 3–4-fold higher and inguinal activities were 4–6-fold higher in females than males.

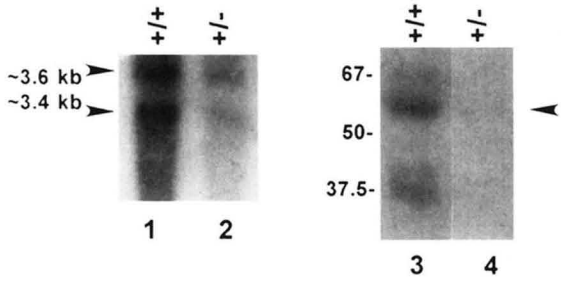

FIG. 7. **LPL mRNA and protein mass in +/+ versus +/− mice.** Adipose tissue RNA was subjected to Northern blotting (*lanes 1* and *2*), and adipose tissue protein was subjected to Western blotting (*lanes 3* and *4*). For *lanes 1* and *2*, 20 μg of total RNA was separated on agarose gels containing formaldehyde. Equal loading and equal RNA integrity for each genotype was verified by analysis of ethidium-stained 28 and 18 S ribosomal RNA intensities before transfer. The LPL message (indicated by the 3.6-kb and 3.4-kb markers) was detected using a random-primed mouse LPL cDNA. For *lanes 3* and *4*, 1 μg of protein was separated by SDS-polyacrylamide gel electrophoresis with the ~55-kDa LPL protein (visible in both lanes at the position indicated by the *arrowhead* to the *right* of *lane 4*) detected using an iodinated chicken anti-bovine LPL antibody. *Numbers* to the *left* of *lane 3* indicate the positions of protein size standards. Results shown for LPL mRNA are representative of comparisons of 5 different pairs of +/+ and +/− animals. Results for LPL protein are representative of comparisons of 3 different pairs of +/+ and +/− animals.

nism does not appear to be operative in our +/− mice. LPL activity (Fig. 6), message, and protein (Fig. 7) are lower in the tissues of heterozygotes, making it likely that our mutation causes loss of function through a decrease in LPL protein. Consistent with this hypothesis, COOH-terminal truncation of human LPL at residue 381, essentially the same site disrupted in mouse LPL in the current study, causes a loss of catalytic activity as well as a decrease in protein mass in transient transfection studies (43). Thus, the phenotype of mice generated by COOH-terminal disruption of LPL may be similar to the phenotype generated by targeting regions of the LPL gene 5′ to the exon 8 site chosen in this study.

Why then do LPL +/− mice have elevated triglycerides in the fasted state on a low fat diet? At least four explanations are possible. First, the disruption of the COOH terminus of the LPL molecule could somehow impair the metabolism of triglyceride-rich lipoproteins as suggested by cell culture studies (12). We do not find evidence of a protein with altered size in +/− tissues to support this hypothesis, but Western blots were done with a single antibody, raising the possibility that an altered protein is produced but not recognized by our antibody. Second, LPL deficiency could affect triglyceride-rich particle composition and impair clearance. Third, LPL deficiency could reduce hepatic reuptake of apo B-containing particles with a net effect of lipoprotein overproduction by the liver (44). Our enzyme assay was not sufficiently sensitive to detect LPL activity in mouse liver, although we have been able to detect very low levels of LPL protein in this tissue by Western blotting (not shown). Fourth, LPL deficiency is likely to increase the concentration of remnant particles in these animals, a possibility suggested by the "shoulders" of particles seen to the right of the triglyceride peak and to the left of the cholesterol peak for males in Fig. 5. Remnants are noncompetitive inhibitors of LPL activity, and even low concentrations of these particles could substantially interfere with VLDL hydrolysis (45).

There are other mouse models of elevated triglycerides. Overexpression of apo CIII in mice results in hypertriglyceridemia (46). This condition is corrected by overexpression of apo E (47), supporting the hypothesis that excess apo CIII displaces apo E from triglyceride-rich lipoproteins, thereby hindering clearance by apo E-dependent mechanisms. Overexpression of apo CI probably operates through a similar mechanism to increase triglycerides (48). Overexpression of apo CII, a cofactor for LPL activity, surprisingly also increases triglycerides, perhaps by interfering with the ability of VLDL to bind glycosaminoglycans (49). Each of these models was generated using human transgenes in mice. Heterozygous LPL-deficient mice have the theoretical advantage of manifesting hypertriglyceridemia that is not dependent on the expression of a protein from another species.

Another potential advantage of our model is the lack of an effect on HDL cholesterol levels. In species with cholesteryl ester transfer protein activity, triglyceride concentrations are inversely related to HDL cholesterol levels, making it difficult to decide whether beneficial effects on vascular disease are due to decreased triglycerides or increased HDL-C. LPL +/− mice suitably backcrossed into the C57BL/6J background could be useful for determining how hypertriglyceridemia alone affects atherosclerosis. In addition, assessing atherosclerotic disease in LPL +/− mice in the setting of diabetes mellitus, ethanol intake, and hypothyroidism, or after crossing these animals with cholesteryl ester transfer protein transgenic mice, with heterozygotes for apo E deficiency, or with heterozygotes for LDL receptor deficiency, could provide insight into whether commonly observed human lipid phenotypes confer atherosclerotic risk.

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