Transgenic Mice Expressing Human Lipoprotein Lipase Driven by the Mouse Metallothionein Promoter

A PHENOTYPE ASSOCIATED WITH INCREASED PERINATAL MORTALITY AND REDUCED PLASMA VERY LOW DENSITY LIPOPROTEIN OF NORMAL SIZE*

(Received for publication, May 5, 1994)

Eva Zsigmond‡, Ekkehard Scheffler‡, Trudy M. Forte§, Rica Potenz‡, Winnie Wu‡, and Lawrence Chan‡

From the *Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, Texas 77030 and the ‡Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, California 74720

We have produced transgenic mice expressing human lipoprotein lipase (LPL) driven by the mouse metallothionein I promoter. We found that integration of the LPL gene construct was associated with a high perinatal mortality. Animals that survived the first 2 weeks of life grew normally afterwards. Compared with controls, transgenic animals had higher post-heparin plasma LPL and tissue LPL activities. Immunoreactive human LPL was detected in their post-heparin plasma but not in controls. Transgenic animals had significantly lower plasma very low density lipoprotein (VLDL) while on a regular laboratory chow. By electron microscopic analysis and nondenaturing polyacrylamide gradient gel electrophoresis, the size and morphology of the plasma VLDL were very similar in transgenic and control animals, which suggests that VLDL particles acted on by the increased tissue LPL in the transgenic animals were mostly taken up by the cell without being released back into circulation. The hypertriglyceridemia and elevated VLDL in response to sucrose feeding were completely abolished in transgenic animals. They also had lower VLDL lipids compared with control animals when they were fed a high-fat, high-cholesterol diet. Feeding the mother of transgenic mice a high-fat diet during pregnancy completely reversed the high perinatal mortality associated with the integrated transgene, which suggests that the deleterious effect of LPL overexpression may be related to the depletion of some essential lipid nutrient.

Lipoprotein lipase (LPL)* is an endothelial enzyme that plays a central role in lipoprotein metabolism (2). LPL is produced mainly in the heart, skeletal muscle, and adipose tissue. It is anchored on the endothelial surface where it comes in contact with the circulating lipoproteins. Studies in vitro indicate that LPL hydrolyzes the triglyceride components of triglyceride-rich lipoproteins; chylomicrons and very low density lipoproteins (VLDL) seem to be the preferred substrates. An inherited deficiency of LPL causes defective chylomicron metabolism, and patients with this genetic defect have symptoms of chylomicronemia and Type I hyperlipoproteinemia (3). Heterozygous LPL deficiency has been identified as a defect in some cases of familial combined hyperlipidemia (4), one of the most common causes of genetic hyperlipidemia (5). Many metabolic disorders such as diabetes, obesity, alcohol ingestion, hypothyroidism, and renal disease are associated with elevated plasma triglycerides. The lipid abnormalities in these disease states are thought to be partly modulated by or secondary to changes in LPL expression (6, 7).

Apart from its well-described function in the catabolism of triglyceride-rich lipoproteins, LPL has been shown to directly bind to the low density lipoprotein (LDL) receptor-related protein (8). The LPL protein may be involved in the cellular uptake of various classes of lipoproteins including chylomicrons/ chylomicron remnants via the LDL receptor-related protein (9); VLDL and LDL by the LDL receptor (10, 11); lipoprotein(a) via enhanced binding to the LDL receptor (12), and possibly VLDL and LDL by a non-LDL receptor-mediated cellular process (13). Hepatic output of apoB-100-containing lipoproteins (14), and the retention of LDL by the subendothelial matrix may also be modulated by LPL (15). LPL is thus a multifunctional protein with far reaching roles in triglyceride metabolism and energy homeostasis in the vertebrate.

The metabolic roles of LPL have largely been inferred from observations in vitro, and in animals subjected to various experimental manipulations. In this study, we aimed at producing an in vivo model of LPL action by generating transgenic mice that overexpress human LPL (hLPL) driven by the mouse metallothionein I promoter. To our surprise, we found that a large number of mice carrying the hLPL transgene died at or shortly after birth. However, feeding the mother a high-fat diet during the last 2 weeks of pregnancy completely reversed the deleterious effect of the transgene on the survival of the transgenic offspring. This was true whether the pregnant mother was a foster mother into which transgene-injected embryos were implanted or was itself a transgenic animal. Using a high-fat diet strategy, we succeeded in generating a total of 10 independent LPL transgenic mouse lines and examined the lipoprotein biochemistry and morphology in transgenic and control animals.

MATERIALS AND METHODS

Preparation of the Human LPL Gene for Microinjection—The complete protein coding region of the hLPL cDNA was contained in a 1.8-kb EcoRI fragment (16). The promoter and transcription initiation site derived from the mouse metallothionein I gene were inserted upstream of the hLPL cDNA and the polyadenylation signal sequence from the
Lipoprotein Lipase Transgenic Mice

human growth hormone gene was ligated to the 3' end of the hLPL cDNA (Fig. 1). The gene construct was subcloned in pGEMBZ, purified by cesium chloride gradient centrifugation, and digested with SalI and NotI, and used for microinjection.

Production of Transgenic Mice—The hLPL gene construct was microinjected into the male pronucleus of fertilized eggs obtained from FVB mice. Injected embryos were reimplanted into pseudo-pregnant human growth hormone gene was ligated to the 3' end of the hLPL by cesium chloride gradient centrifugation, and digested with SalI and cDNA (Fig. 1). The gene construct was subcloned in pGEMBZ, purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.

Table 1

<table>
<thead>
<tr>
<th>Founders</th>
<th>Number implanted</th>
<th>Number of pups born and survived</th>
<th>Number of transgenics</th>
<th>% of transgenics that were born and survived of total number of embryos implanted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1460</td>
<td>26</td>
<td>23</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>32</td>
<td>0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Production of Recombinant Human LPL and Production of Chicken Anti-LPL Antibody**—In order to produce sufficient amounts of highly purified hLPL for antibody production, we have cloned the hLPL cDNA in the expression vector pE14. This vector contains human cytoenerulovirus 5' sequences and SV40 3' sequences, and contains the glutamine termination signal from the human growth hormone gene (0.6 kb, stippled box) and the transcription termination initiation site (1.7 kb, stippled box) were derived from plasmid pMThGIII (30). Solid lines represent polynucleotide sequences. The hLPL transgene construct was subcloned in pGEMBZ and purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.

**Production of Recombinant Human LPL and Production of Chicken Anti-LPL Antibody**—In order to produce sufficient amounts of highly purified hLPL for antibody production, we have cloned the hLPL cDNA in the expression vector pE14. This vector contains human cytoenerulovirus 5' sequences and SV40 3' sequences, and contains the glutamine termination signal from the human growth hormone gene (0.6 kb, stippled box) and the transcription termination initiation site (1.7 kb, stippled box) were derived from plasmid pMThGIII (30). Solid lines represent polynucleotide sequences. The hLPL transgene construct was subcloned in pGEMBZ and purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.

**Production of Recombinant Human LPL and Production of Chicken Anti-LPL Antibody**—In order to produce sufficient amounts of highly purified hLPL for antibody production, we have cloned the hLPL cDNA in the expression vector pE14. This vector contains human cytoenerulovirus 5' sequences and SV40 3' sequences, and contains the glutamine termination signal from the human growth hormone gene (0.6 kb, stippled box) and the transcription termination initiation site (1.7 kb, stippled box) were derived from plasmid pMThGIII (30). Solid lines represent polynucleotide sequences. The hLPL transgene construct was subcloned in pGEMBZ and purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.

**Production of Recombinant Human LPL and Production of Chicken Anti-LPL Antibody**—In order to produce sufficient amounts of highly purified hLPL for antibody production, we have cloned the hLPL cDNA in the expression vector pE14. This vector contains human cytoenerulovirus 5' sequences and SV40 3' sequences, and contains the glutamine termination signal from the human growth hormone gene (0.6 kb, stippled box) and the transcription termination initiation site (1.7 kb, stippled box) were derived from plasmid pMThGIII (30). Solid lines represent polynucleotide sequences. The hLPL transgene construct was subcloned in pGEMBZ and purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.

**Production of Recombinant Human LPL and Production of Chicken Anti-LPL Antibody**—In order to produce sufficient amounts of highly purified hLPL for antibody production, we have cloned the hLPL cDNA in the expression vector pE14. This vector contains human cytoenerulovirus 5' sequences and SV40 3' sequences, and contains the glutamine termination signal from the human growth hormone gene (0.6 kb, stippled box) and the transcription termination initiation site (1.7 kb, stippled box) were derived from plasmid pMThGIII (30). Solid lines represent polynucleotide sequences. The hLPL transgene construct was subcloned in pGEMBZ and purified by cesium chloride gradient centrifugation. Digestion with SalI and NotI resulted in the release of a 4.1-kb linear DNA fragment that was gel purified and microinjected into fertilized eggs from FVB mice. Transgenic mice were identified by Southern blot analysis of tail DNA. mMT-1, mouse metallothionein I.
FIG. 2. Reverse transcriptase-PCR analysis of human LPL expression in transgenic mouse tissues. The drinking water of transgenic and nontransgenic mice was supplemented with 25 mM ZnSO₄ for 5 days. Tissues were obtained from mice and immediately frozen in liquid N₂. Total RNA was prepared from each tissue by the RNAzol B method (Biotech Laboratories, Inc.) as described under "Materials and Methods." In order to synthesize the first strand cDNA, 8 μg of total RNA was incubated with 10 pmol of the 5' and 3' primers in F₂, first strand buffer (BRL BH 1132) at 68 °C for 7 min. Following a brief cooling period, 10 μl dNTP, 20 units of RNasin, and 50 units of reverse transcriptase were added to the reaction mixture in a total volume of 20 μl. After a 30-min incubation at 42 °C, DNA amplification was performed according to the GeneAmp protocol (Perkin-Elmer). Samples were incubated at 94 °C for 1 min and 58 °C for 5 min for 30 cycles. For each tissue sample, a 20-μl aliquot of the PCR product was fractionated on a 5% denaturing polyacrylamide gel. In both transgenic mouse lines, amplification of RNA samples from liver, adipose tissue, heart, muscle, and brain consistently resulted in the recovery of the expected 314-base pair fragment (indicated by the triangle). RNA isolated from control mice did not show this band (data not shown).

FIG. 3. Tissue lipase activity in transgenic and control mice fed a regular chow diet. Mice were treated with zinc supplemented water (25 mM) for 5 days. In order to measure LPL activity in postheparin plasma, mice were injected intraperitoneally with 500 units/kg body weight heparin sulfate. After 20 min mice were anesthetized with Avertin and blood was collected from the tail vein. Duplicate 25-μl aliquots of plasma were used to measure lipolytic activity (19). Lipase activity was expressed as milliunits/ml (1 milliunit = 1 nmol of free fatty acid released per min). LPL activity was assayed in 50 μg of tissue collected from transgenic and control mice. Tissues were homogenized at 4 °C in 0.2 ml of detergent solution (2 mg/ml sodium deoxycholate, 0.08 mg/ml Nonidet P-40, 0.05 mg/ml heparin, 10 mg/ml bovine serum albumin, 0.25 M sucrose in 0.225 μl Tris-HCl buffer, pH 8.5) as previously described by Iverius et al. (19). After extraction, tissue samples were assayed for lipase activity as reported under "Materials and Methods." Bars represent mean ± S.E.; n = 8 for tissue lipase activity; n = 7 for postheparin plasma lipase activity. Student's t test was performed to assess differences between lipase activity in transgenic and nontransgenic mice. * = p < 0.05.

FIG. 4. Immunoblot analysis of post-heparin plasma. Plasma was obtained from control and transgenic mice 15 min after heparin injection (PH), processed as described under "Materials and Methods." Immunoblot analysis was performed by a chemiluminescence protocol (DuPont). The antisera used was polyclonal chicken antiserum prepared against recombinant human LPL (rhLPL) produced in Chinese hamster ovary cells as described under "Materials and Methods." Transgenic mice were identified by Southern blot analysis of tail DNA.

After microinjecting a large number of embryos, it became evident that the number of pups born after implantation in a foster mother was much smaller than a control gene (apolipoprotein A-II) (24) microinjected at the same time in our laboratory. Furthermore, compared to the control construct, a high proportion of the LPL transgenic pups died within 2–3 days of
### Plasma Lipid and Lipoprotein Concentrations in Transgenic and Control Mice Fed Regular Chow

Values represent mean ± S.E. for n, number of mice in three separate experiments (experiments 1–3). Differences between transgenic and control mice were tested by Student's t test.

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Total Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol (µg/total fraction)</td>
<td>Triglyceride (µg/total fraction)</td>
<td>Cholesterol (µg/total fraction)</td>
<td>Triglyceride (µg/total fraction)</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>5.72 ± 0.55</td>
<td>32.76 ± 3.06</td>
<td>27.35 ± 1.30</td>
<td>18.20 ± 1.75</td>
</tr>
<tr>
<td>Control</td>
<td>14.04 ± 2.06</td>
<td>66.84 ± 4.96</td>
<td>32.67 ± 4.60</td>
<td>22.15 ± 0.90</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>1.26 ± 0.66</td>
<td>16.55 ± 1.03</td>
<td>26.98 ± 2.36</td>
<td>28.73 ± 0.58</td>
</tr>
<tr>
<td>Control</td>
<td>6.37 ± 2.15</td>
<td>38.01 ± 5.58</td>
<td>29.60 ± 4.67</td>
<td>30.11 ± 3.40</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>3.64 ± 0.58</td>
<td>26.53 ± 4.49</td>
<td>17.80 ± 3.00</td>
<td>46.63 ± 7.89</td>
</tr>
<tr>
<td>Control</td>
<td>6.97 ± 1.01</td>
<td>45.92 ± 11.34</td>
<td>22.90 ± 3.46</td>
<td>48.04 ± 11.94</td>
</tr>
</tbody>
</table>

* Means ± S.E.  
\(^b p < 0.05.

**FIG. 5. Fractionation of plasma lipoprotein by FPLC.** Transgenic and nontransgenic control mice were fasted for 16 h and anesthetized by intraperitoneal injection of Avertin. Plasma samples (200 µl) from individual mice were subjected to gel filtration chromatography using a Beckman System Gold HPLC/FPLC system with two Superose 6 columns connected in series. Samples were eluted with 1 mM EDTA, 154 mM NaCl, and 0.02% NaN₃ (pH 8.2) at a rate of 0.5 ml/min at room temperature. Forty fractions were collected at 1-min intervals using a Gilson microfractionator. FPLC fractions (150 µl) were assayed for total cholesterol and triglyceride content using enzymatic assay kits from Sigma Diagnostics.

Birth (Table I). The high perinatal mortality rate was also evident when the founder animals were cross-bred with normal FVB mice. A large number of the F1 transgenic offspring also died within 2 weeks of birth. This was true for both founder lines which, by Southern blot analysis, were shown to have different transgene integration sites. Therefore, the integration of the hLPL transgene resulted in lower survival rates for two independent transgenic lines. In subsequent generations we obtained a few homozygous animals by cross-breeding the heterozygous mice. However, cross-breeding two homozygous animals consistently failed to produce viable offspring in both lines. We observed that either pregnancies terminated spontaneously before any pups were delivered or newborn transgenic pups died within 24 h of birth. Of the two surviving LPL transgenic mouse lines, line 1 contained 10–15, and line 2, 3–5 copies of the transgene per mouse genome.

**Transgene mRNA Expression in Transgenic Mice**—The structure of LPL is highly conserved among mammals (25–27) and there is significant cross-hybridization of the hLPL cDNA probe to mouse LPL mRNA on Northern blot analysis. We have therefore assessed the expression and tissue distribution of transgene-specific mRNA by reverse transcriptase-PCR. Total RNA
was extracted from various tissues of transgenic and control animals. RNA samples were amplified by reverse transcriptase-PCR as described under "Materials and Methods," and PCR products were fractionated on 5% polyacrylamide gels. By this technique, the transgene-specific PCR product was observed in transgenic mouse liver, adipose tissue, heart, kidney, spleen, testis, lung, adrenal, muscle, and brain (Fig. 2). No expression was detected in the small intestine of the animals. A PCR product was produced from RNAs extracted from any of the same tissues from nontransgenic control mice (data not shown).

**Plasma and Tissue Lipoprotein Lipase Activity in Transgenic and Control Mice**—Since the transgene construct was under the direction of a heavy metal-inducible metallothionein promoter, we compared the post-heparin plasma LPL activity in transgenic and control mice that were maintained on a ZnSO₄-enriched drinking water for 5 days. In the experiment depicted in Fig. 3, right panel, the post-heparin LPL activity in transgenic mice was higher than that in control animals by 24%. We next measured LPL activity extracted from the major tissues that express hLPL (Fig. 3, left panel). In control animals, adipose tissue and heart expressed high LPL activity, whereas the activity in muscle was approximately one-third that in these tissues. The barely detectable LPL activity extracted from the liver of control mice was statistically not different from zero. In transgenic mice, tissue LPL activity was higher in heart (317% of control), muscle (216% of control), and liver (225% of control), but not in adipose tissue (96% of control). However, because of the considerable variation in LPL activity extracted from these tissues in both control and transgenic animals, the increase in LPL activity was statistically significant only in heart extracts.

**Immunoreactive Lipoprotein Lipase in Post-heparin Plasma in Transgenic Mice**—We performed immunoblot analysis of transgenic and control mouse plasma before and after heparin treatment. We used a monospecific chicken polyclonal anti-serum against homogeneous purified recombinant hLPL produced in *vitro* for immunodetection. Before heparin administration, we did not detect any immunoreactive LPL (data not shown). Within 15 min of intraperitoneal heparin, an immunoreactive LPL band was clearly identified in transgenic plasma but not in nontransgenic controls (Fig. 4).

**Plasma Lipoprotein Levels in Transgenic and Control Mice**—Lipoprotein lipase is the major enzyme required for the metabolism of triglyceride-rich lipoproteins. We examined the plasma lipid and lipoprotein profiles in transgenic and control animals. Because these experiments were carried out over a span of over 3 years, there was considerable variation in the lipid and lipoprotein parameters. However, all experiments included control and transgenic animals receiving diet treatment at the same time. The findings were always consistent over the course of the 3-year period.

We first examined the plasma lipids and lipoproteins while the animals were on a regular laboratory chow diet. There is no difference in the total plasma triglyceride and cholesterol between transgenic and control animals in the first two experiments (Table II). In the third experiment, using a new transgenic line we obtained subsequently (see below), the total plasma cholesterol and triglyceride were significantly lower in transgenic mice compared to controls (Table II).

We next fractionated the plasma lipoproteins by FPLC chromatography on a Superose-6 column. This technique gave good separation of mouse plasma into VLDL, LDL, and HDL (Fig. 5). The lipids in each lipoprotein fraction were determined. Confirming previous studies on other mouse strains, we found that HDL is the major species of lipoproteins in both control and transgenic mice. Transgenic mice had significantly lower plasma VLDL cholesterol (40% of control) and triglyceride (49% of control) compared to nontransgenic littermate controls (Table II). The plasma LDL and HDL lipids were not significantly different in the two groups of animals. These observations were confirmed in the second line of transgenic mice. In this line, the plasma VLDL cholesterol (20% of control) and triglyceride (44% of control) were both lower than controls (Table II). In a third line which shows higher level of expression obtained over 1 year later (see below), the plasma VLDL cholesterol (52% of control) and triglyceride (58% of control) were again lower than controls.

**Electron Microscopic and Gel Analysis of Purified Plasma Lipoproteins from Transgenic and Control Mice**—Electron microscopic evaluation of isolated lipoprotein fractions from chow-fed transgenic and control mice revealed that intermediate density lipoproteins were not present in either group of animals. Electron microscopy further revealed great similarity in the morphology and size distribution of VLDL, LDL, and HDL in transgenic and control mice. As seen in Fig. 6A, VLDL from transgenic and control mice are heterogeneous sized round particles; the mean particle diameter for transgenic VLDL is 51.4 ± 15.6 nm while that of controls is 55.4 ± 17.1 nm (Table III). Because of the large standard deviation of mean particle diameters, there is essentially no difference in size between the two groups of animals. However, control mouse VLDL are twice as many particles larger than 72.0 nm (19.3% as transgenic animals (10.9%), which accounts for the somewhat larger mean diameter of control VLDL. Transgenic mouse LDL and HDL consist of round particles (Fig. 6A) (23.5 ± 4.4 and 9.9 ± 1.7 nm, respectively) which were similar in size to those of control mice (23.5 ± 4.7 and 9.9 ± 1.6 nm, respectively).

Nondenaturing gel distributions of transgenic and control mouse VLDL, LDL, and HDL (Fig. 6B) support the similarity of lipoprotein profiles noted on electron microscopy (summarized in Table III).

**Effect of High-fat Diet on Plasma and Tissue Lipoprotein Lipase Activity and Plasm Lipoproteins**—We tested the effect of a high-fat, high-cholesterol diet on the plasma and tissue LPL activities. Control and transgenic mice were fed a special diet containing 1.25% cholesterol and 11% fat for 4 weeks. Compared to chow-fed mice, plasma post-heparin LPL activity was increased 4-5-fold in both control and transgenic animals and the average LPL activity was higher in the transgenic animals, being 179% of control (Fig. 7, right panel). However, there was marked variability in the response and the difference between the two types of animals was not statistically significant. Similarly, feeding high-fat diets also stimulated LPL activities extracted from various tissues 2-4 fold in liver, fat, and heart (Fig. 7, left panel) in both control and transgenic animals.
FIG. 6. A, electron micrographs of lipoproteins. Negatively stained lipoprotein fractions from control mice (a, VLDL; b, LDL; c, HDL) and transgenic mice (d, VLDL; e, LDL; f, HDL) were examined under an electron microscope. The bar markers represent 100 nm. B, nondenaturing polyacrylamide gradient gel (4–30%) profiles of transgenic and control lipoprotein fractions. a and b, control and transgenic VLDL, respectively; c and d, control and transgenic LDL, respectively; and e and f, control and transgenic HDL, respectively. g, standards, bands used to calibrate the gel are indicated at the right.

The effect of the high-fat, high-cholesterol diet on the plasma lipoproteins of transgenic animals was also examined. With a lower basal VLDL in transgenic animals, they responded with a much greater increase in VLDL (averaging a 39-fold increase in VLDL-cholesterol in the two experiments depicted in Table IV) than nontransgenic controls (which averaged a 16-fold increase). However, there was marked variability in the plasma lipids and lipoproteins in both transgenic and control animals in response to the diet. Although VLDL triglyceride and cholesterol levels were lower in transgenic animals, including a high expression line obtained a year later (experiment 3, see below), compared to controls, the difference did not reach statistical significance in three separate experiments (Table IV).

**Effect of High Sucrose Diet on Plasma Lipoproteins**—Endogenous hypertriglyceridemia can be induced in animals by high carbohydrate feeding (28, 29). We tested the effect of hLPL expression in transgenic mice on the endogenously produced VLDL in response to a high sucrose diet (Table V). Control mice fed the special diet for 7 days developed a marked hypertriglyceridemia with a 3-fold increase in total plasma triglyceride. This increase in triglyceride was all accounted for by an elevated plasma VLDL; VLDL triglyceride went up about 6-fold and VLDL cholesterol went up about 3½-fold. In transgenic animals fed the same diet, the increase in total plasma triglyceride was totally abolished. Similarly the VLDL response was also completely eliminated so that the VLDL lipids were not different from chow-fed controls. Therefore, the overexpression of hLPL was quite effective in catabolizing the endogenously synthesized VLDL and completely prevented high carbohydrate-induced hyperlipidemia in mice.

**Effect of High-fat Diet on Perinatal Mortality**—The cause of the high perinatal mortality (see Table I) among transgenic mice is unknown. However, since the plasma VLDL triglycerides are reduced to 45–60% of control in transgenic mice on regular laboratory chow, we reasoned that it is possible that during the intrauterine and early postnatal period, the trans-
genic pups were depleted of lipid substrates and some tissues were actually "starving" from lack of circulating lipid nutrients. To test whether LPL-induced substrate depletion was a plausible explanation for the high perinatal mortality, we studied the effect of a high-fat, high-cholesterol diet on the outcome of pregnancy in transgenic and control animals. We found that the lethal effects of the transgene were completely reversed by feeding the mother the high-fat diet during the last 2 weeks of pregnancy (Table VI, A). The perinatal mortality was reduced by >90% and the proportion of transgenic offspring that survived the perinatal period was more than doubled by this manipulation.

The initial statistics on the perinatal mortality of the progeny were derived from only two transgenic mouse lines. These were independent transgenic lines having different sites of transgene integration revealed by Southern blots. It is possible, although highly unlikely, that in both lines, the two sites of integration disrupted some functions which coincidentally also impaired survivability that could be reversed by high-fat diet feeding. We further investigated whether we could generate new founder lines using this high-fat diet strategy, and whether survival at the founder level was diet-related. We repeated all our transgenic experiments using the same metallothionein-hLPL construct and microinjected and implanted another 420 embryos (Table VI, B). The foster mothers were fed either regular chow or a high-fat diet. We observed a major difference in the rate of successfully generating transgenic founder animals from mothers fed the two diets: for the chow-fed foster mothers, we generated a single transgenic mouse from 240 embryos implanted, a success rate of 0.4%; for the high-fat diet fed foster mothers, we obtained 7 transgenic founder animals from 180 embryos implanted, a success rate of 3.9%. To ensure that the observed difference was not a result of a difference in transgenic founder animal survival induced by the two diets but unrelated to the transgene construct, we simultaneously performed a control experiment using an unrelated transgene (a chloramphenicol acetyltransferase gene driven by an avian skeletal muscle actin promoter, Table VI, B). It is evident that for the control transgene the success rates of generating transgenic founder animals are the same (2.6 and 2.9% of embryos implanted for chow- and high-fat diet fed mothers, respectively) for the two diets fed to the mother. When pregnant founder LPL transgenic animals were fed chow or high-fat diets, the birth statistics for the progeny (F1) observed in the initial two lines shown in Table I were again seen in these new transgenic lines with a high neonatal mortality in the chow-fed animals only (data not shown). Therefore, the diet effect is transgene-specific and affects multiple independent founder animals and their progeny.

**Characteristics of New Transgenic Mouse Lines Obtained with the New Strategy**—Using the new strategy, we obtained eight new transgenic founder animals, one from a chow-fed foster mother, seven from high-fat diet fed foster mothers (Table VI, B). All except two of these lines displayed LPL activities and lipoprotein profiles generally similar to the first two lines (Table I) obtained with a regular chow diet. However, two of the seven new lines obtained from the high-fat diet fed mother expressed much higher LPL activities. As shown in Fig. 8, the tissue LPL activities of the initial lines were up to ~160% of control (in heart), whereas the levels in the new transgenic line examined were 200–300% higher. We repeated the lipoprotein analyses on the progeny of these new transgenic lines under various dietary conditions. In Tables II and IV, experiment 3 refers to the results obtained from one of the two high expression transgenic mouse lines obtained with this new strategy. These high expression lines displayed a similar or

![Fig. 7. Tissue lipase activity in transgenic and control mice fed a high-fat diet.](image)

**Table IV**

Plasma lipid and lipoprotein concentrations in transgenic and control mice fed a high-fat diet

<table>
<thead>
<tr>
<th>n</th>
<th>VLDL µg/total fraction*</th>
<th>LDL µg/total fraction*</th>
<th>HDL µg/total fraction*</th>
<th>Total plasma mg/dl*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>5</td>
<td>117.56 ± 21.40</td>
<td>33.18 ± 5.05</td>
<td>31.93 ± 7.81</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>135.27 ± 28.74</td>
<td>47.98 ± 5.94</td>
<td>49.91 ± 9.89</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>8</td>
<td>132.54 ± 24.83</td>
<td>21.23 ± 4.28</td>
<td>66.80 ± 5.44</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>150.89 ± 13.56</td>
<td>29.05 ± 8.11</td>
<td>55.92 ± 6.71</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>3</td>
<td>112.98 ± 21.33</td>
<td>17.78 ± 0.98</td>
<td>60.17 ± 7.97</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>165.76 ± 45.32</td>
<td>18.05 ± 3.32</td>
<td>81.73 ± 26.07</td>
</tr>
</tbody>
</table>

*Means ± S.E.
more pronounced lowering of the plasma VLDL levels in response to a regular chow diet or high-fat, high-cholesterol diet (Tables II and IV).

**DISCUSSION**

Lipoprotein lipase plays a major role in the metabolism of triglyceride-rich lipoproteins. We have created transgenic mice expressing hLPL to examine this function of LPL, and to see if other phenotypic changes might result from LPL overexpression. Because of the unexpected high perinatal mortality of transgenic animals, it took a massive effort before we were able to establish the first two independent mouse lines (Table I). After we discovered that high-fat feeding markedly improved survival of the transgenic animals, we obtained eight additional founder animals and repeated all the experiments in several of the new lines. There are two interesting findings revealed by our analysis of the hLPL transgenic mice: the hyperlipidemia affecting VLDL selectively and the high perinatal mortality.

In animals on a regular chow, we found that the plasma post-heparin LPL activity was about 24% higher in the transgenic animals obtained in a conventional way. It was ~120% higher in a high-expression line obtained by feeding the foster mother of the founder embryo a high-fat diet. In this line, high LPL expression was also observed in liver, adipose, heart, and muscle. The LPL transgene construct is under the direction of the mouse metallothionein I promoter. The fairly widespread tissue distribution (Fig. 2) of transgene-specific hLPL transcripts is consistent with the wide tissue specificity of this promoter (30).

Total plasma triglyceride and cholesterol were lower in the high expression transgenic mice compared to controls when they were on a regular laboratory chow. The lowering was moderate although HDL, the major lipoprotein species in mice, is not affected by LPL overexpression. Plasma lipoprotein analysis (Fig. 4 and Table II) in all transgenic animals showed a consistently decreased VLDL triglyceride and cholesterol. Experiments on live animals in the past that suggest an important functional role of LPL in vivo include the following: (i) the release of endothelial LPL by heparin injection resulted in a marked lowering (clearing) of plasma triglycerides (31-35); (ii) the injection of anti-LPL antibodies in vivo resulted in a buildup of plasma triglycerides (36, 37); and (iii) the occurrence of chylomicronemia in patients with hereditary LPL deficiency (3). The consistent presence of hyperlipidemia with respect to plasma VLDL in the transgenic animals overexpressing hLPL is direct in vivo evidence that LPL is involved in the metabolism of plasma VLDL. Interestingly, the other lipoprotein fractions, LDL and HDL, were unchanged in the transgenic animals.

Overexpression of hLPL appears to have no major effect on particle size distribution and morphology of mouse LDL and HDL. This suggests that although LPL activity is higher in the transgenic mouse than control, elevated activity does not result in the formation of surface remnants in the LDL such as has been observed in heparin-induced lipolysis in human subjects (38). Also, in the latter study, increased LPL activity was associated with the formation of smaller sized HDL, whereas in the transgenic and control mice the size of HDL remains constant, which is consistent with the FPLC profiles and lipid compositions of transgenic and control HDL. There is recent evidence that the LPL-mediated binding of LDL and VLDL to extracellular heparin sulfate proteoglycans is followed by internalization of the lipoproteins, mainly through the classic LDL receptor pathway (11). It is possible that in transgenic mice, this LPL-mediated process is accelerated and most of the attached VLDL particles were never released into circulation before they were taken up by the cell. If this is the explanation for the much lower plasma VLDL concentration, the size of the VLDL particles is not expected to undergo any major change in the LPL-transgenic mice. Nonetheless, despite the similar average

---

**Table V**

<table>
<thead>
<tr>
<th>n</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Total plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>Cholesterol</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>---</td>
<td>------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Transgenic (high CHO)</td>
<td>5</td>
<td>6.63 ± 1.26**</td>
<td>65.95 ± 17.87**</td>
<td>33.39 ± 5.76</td>
</tr>
<tr>
<td>Control (high CHO)</td>
<td>6</td>
<td>24.49 ± 2.80</td>
<td>263.43 ± 22.02</td>
<td>41.63 ± 6.79</td>
</tr>
<tr>
<td>Control (chow)</td>
<td>4</td>
<td>6.97 ± 1.01</td>
<td>45.92 ± 11.34</td>
<td>22.90 ± 3.46</td>
</tr>
</tbody>
</table>

*Means ± S.E.; **p < 0.05; ***p < 0.01, compared to controls fed the high-carbohydrate diet. This experiment was done with experiment 3 in Table II and the same chow controls were used in both experiments.

---

**Table VI**

**Effect of a high-fat diet during pregnancy on the birth statistics of LPL-transgenic mice**

<table>
<thead>
<tr>
<th>LPL-transgenic</th>
<th>Control-non-transgenic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>High fat</td>
</tr>
<tr>
<td>Number of embryos implanted</td>
<td>240</td>
</tr>
<tr>
<td>Number of embryos born</td>
<td>28</td>
</tr>
<tr>
<td>Number of transgenic pups</td>
<td>1</td>
</tr>
</tbody>
</table>

*These are nontransgenic control mice of the same strain (FVB) maintained in the same facility at the same time.  
*b Animals that died within 2 weeks of birth.  
*c The expected proportion of transgenic pups is 50% because the founders are hemizygous for the transgene.  
*d The LPL and control transgenes were microinjected and implanted by the same person.  
*e Control transgene used is the chloramphenicol acetyltransferase gene driven by the avian skeletal muscle actin promoter.

---

...
expected. Since it was observed in multiple independent lines and observed in all transgenic animals fed a high carbohydrate diet at both the founder and progeny levels, we have ruled out a possible causes for the lethality of LPL overexpression in transgenic animals. Under "Materials and Methods," there is a consistent and major difference may be more efficiently cleared in the transgenic mice. These large particles are consistent with the observed higher VLDL triglyceride in control animals compared with transgenic animals suggests that such particles may be more efficiently cleared in the transgenic mice.

The response of transgenic and control mice to a high-fat diet is highly variable. In the low expression LPL transgenic lines, the difference in plasma lipoprotein lipids between transgenic and control animals did not reach statistical significance (Fig. 7). However, with the two high expression lines produced by the chow-fed mothers and the high expressor animals were from a founder produced by a high-fat diet fed mother. The techniques are as described under "Materials and Methods." VLDL size in transgenic and control animals, control mice had almost twice as many large VLDL, i.e. greater than 72 nm, as the transgenic animals. These large particles are consistent with the observed higher VLDL triglyceride in control animals seen in Fig. 5. The presence of very large VLDL in control as compared with transgenic animals suggests that such particles may be more efficiently cleared in the transgenic mice.

The high perinatal mortality of transgenic mice was unexpected. Since it was observed in multiple independent lines and at both the founder and progeny levels, we have ruled out a position (i.e. integration) effect of the transgene. There are two possible causes for the lethality of LPL overexpression in transgenic mice: (i) LPL overexpression produced toxic side products, because in addition to triglyceride hydrolysis, LPL also has other activities, e.g. it is also a phospholipase and toxic levels of lysolecithin might build up in the transgenic animals, (ii) LPL overexpression merely depleted the circulating lipoproteins such that the tissues were actually starving because of the resulting hypolipidemia. Although we have not provided direct proof for the second explanation, the fact that high-fat diet feeding during the last 2 weeks of pregnancy completely reversed the high perinatal mortality of transgenic offspring (see Table VI) essentially ruled out toxic products as the cause of this deleterious effect of LPL overexpression.

Since the initial submission of this manuscript, Shimada et al. (39) reported the production of transgenic mice overexpressing hLPL. They used a construct containing a hLPL cDNA with a chicken b-actin promoter and a rabbit b-globin polyadenylation signal in the 3' end. Despite the use of different construct designs and different strains of mice, the observations on lipid profiles were generally similar. One major difference in the two studies is the lack of information on the comparative mortality rates of the LPL transgenic mice in the previous study. If we assume that Shimada et al. (39) did not observe a markedly reduced survival rate in the transgenic animals during the neonatal period, we can conclude that the high perinatal mortality affecting the transgenic mice in this study may be construct-specific. It is possible that the different patterns of tissue expression account for the presumed difference in perinatal mortality. The marked protective effect of a high-fat diet during pregnancy observed in this study was not evaluated by Shimada et al. (39). In another recent study using a hLPL construct driven by a cytomegalovirus promoter, Liu et al. (40) did not detect any effect of hLPL transgene expression on viability, although the effect of a high-fat diet was not evaluated. These different results again support the conclusion that the viability differences observed are promoter or construct-specific. Furthermore, the observed difference in survivability under the two dietary conditions presented in Tables I and VI suggests that the use of the metallothionein I-driven hLPL construct in a chow-fed mother causes intrauterine death of the high expression animals such that only low expression lines are selected for survival. High expression animals survived only when the deleterious effect of the transgene was reversed by the high-fat diet.

Acknowledgments—We thank S. Tobola for expert secretarial assistance, and Laura Knoff for assistance with the electron microscopic analysis.

REFERENCES

FIG. 8. Tissue and post-heparin plasma lipase activity in control, transgenic "low" expressor and "high" expressor mice. The low expressor animals were obtained from a founder produced from chow-fed mothers and the high expressor animals were from a founder produced by a high-fat diet fed mother. The techniques are as described under "Materials and Methods."
Lipoprotein Lipase Transgenic Mice