Adenovirus-mediated Rescue of Lipoprotein Lipase-deficient Mice

LIPOLYSIS OF TRIGLYCERIDE-RICH LIPOPROTEINS IS ESSENTIAL FOR HIGH DENSITY LIPOPROTEIN MATURATION IN MICE

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Lipoprotein lipase (LPL) is the rate-limiting enzyme for the hydrolysis of triglycerides and the subsequent uptake of free fatty acids in extrahepatic tissues. Deficiency of LPL in humans (Type I hyperlipoproteinemia) is associated with massive chylomicronemia, low high density lipoprotein (HDL) cholesterol levels, and recurrent attacks of pancreatitis when not controlled by a strict diet. In contrast to humans, homozygous LPL knock-out mice (L0) do not survive suckling and die between 18 and 24 h after birth. In this study, an adenovirus-based protocol was utilized for the transient expression of LPL during the sucking period in an effort to rescue L0 mice. After a single intraperitoneal injection of 5 × 10⁵ plaque-forming units of LPL-expressing virus immediately after birth, more than 90% of L0 mice survived the first days of life. 3% of L0 mice survived the entire sucking period and lived for up to 20 months, although LPL activity in mouse tissues and postheparin plasma was undetectable in all animals after 6 weeks of age. Adult LPL-deficient mice were smaller than their littermates until 2-3 months of age and exhibited very high triglyceride levels in the fed (4997 ± 1102 mg/dl) and fasted state (2007 ± 375 mg/dl). Plasma total cholesterol levels, free fatty acids, and ketone bodies were elevated in L0 mice, whereas plasma glucose was normal. Most strikingly, L0 mice lacked apoA-I-containing preβ-HDL particles as well as mature HDL resulting in undetectable HDL cholesterol and HDL-apoA-I levels. HDL deficiency in plasma was evident despite normal apoA-I mRNA levels in the liver and normal apoA-I protein levels in plasma, which were predominantly found in the chylomicron fraction. The absence of preβ-HDL and mature HDL particles supports the concept that the lipolysis of triglyceride-rich lipoproteins is an essential step for HDL maturation.

The major function of LPL is the enzymatic cleavage of acyl-glycerol esters in triglycerides (TG) of very low density lipoproteins (VLDL) and chylomicrons. Following its synthesis in parenchymal cells such as adipocytes and muscle cells, the enzyme is translocated and bound to the intimal side of the capillary endothelium by its interaction with sulfated glucosaminoglycans (for a review, see Refs. 1–3). Free fatty acids (FFA), the products of plasma TG hydrolysis, are absorbed by the underlying tissue for storage (adipose tissue) or energy production (muscle) (5). Besides this important enzymatic function, LPL has also been shown to act as a ligand or bridging factor for the receptor-mediated cellular uptake of various lipoproteins (6–8). Additionally, LPL facilitates the selective uptake of lipids and lipophilic vitamins (9–11). Both enzymatic and nonenzymatic LPL-mediated processes greatly affect the metabolism of plasma lipoproteins and energy homeostasis in all vertebrates.

LPL deficiency (type I hyperlipoproteinemia) (12) is a rare autosomal, recessively inherited disease characterized by elevated plasma TG levels, low plasma total cholesterol (TC) levels, and drastically decreased HDL cholesterol (HDL-C) concentrations. Besides these lipid abnormalities, the disorder is associated with the development of hepato- and splenomegaly, eruptive xanthomas, lipemia retinalis, and abdominal pain on a standard diet, which leads to frequent attacks of pancreatitis. The profoundly reduced HDL-C levels in LPL-deficient individuals are based on the role of LPL in HDL biogenesis. In this multistep process, lipid-poor or lipid-free HDL precursors (preβ-HDL and apoA-I, respectively) are produced and secreted by hepatocytes or enterocytes. Alternatively, these particles are also produced by the LPL-mediated lipolysis of chylomicrons and VLDL or the HDL modification by phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) (13). HDL precursor particles accept phospholipids and cholesterol from cells through an efflux mechanism that involves the ATP binding cassette transporter 1 (ABCA1) (15–18). Subsequently, these particles are converted into mature, large, and spherical HDL-3 and HDL-2 by a process that involves the esterification of cholesterol by lecithin:cholesterol acyltransferase (19), the acceptance of surface remnants from TG-rich lipoproteins (20), and the fusion of HDL particles. The latter two processes are mediated by PLTP (21, 22). According to this pathway, the lack of TG lipolysis in LPL-deficient individuals impairs the generation of HDL precursors and prevents their maturation. More indirectly, the pronounced hypertriglyceri-
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Isolation and Analysis of LPL—Lipoproteins were isolated by fast protein liquid chromatography (FPLC) using an Amer-...
Chemicals, Neuss, Germany) immediately after the blood was collected. If not otherwise stated, results are given as means ± S.D. Statistical significance was tested by using the two-tailed Student’s t test.

Histologic Analysis—After killing the mice with Isofluran (Amer sham Pharmacia Biotech and Upjohn), various tissues were excised and prepared for analysis. Liver, heart muscle, skeletal muscle, kidney, brain, and spleen were formalin-fixed, embedded in paraffin wax by conventional techniques, hematoxylin-eosin-stained, and examined as previously described (40).

RESULTS

Ad-LPL Expression and Survival of L0 Mice—In an attempt to rescue L0 mice from neonatal death, complete litters of newborn mice from 134 L1 × L1 matings were intraperitoneally injected with LPL-expressing adenovirus (Ad-LPL) immediately after birth (2–8 h). The optimal dose of Ad-LPL was determined by injecting four different virus concentrations: 5 × 10⁸, 1 × 10⁹, 5 × 10⁹, and 8 × 10⁹ pfu, respectively. The best results were obtained with an Ad-LPL concentration of 5 × 10⁹ pfu, which was then used in all subsequent experiments. As depicted in Fig. 1, Ad-LPL injection markedly increased the survival rate of L0 mice. Whereas all untreated L0 mice died within 24 h after birth, 97% of treated L0 animals were alive after this time point. During suckling, the mortality of treated animals increased. After 1 week, 35% of the L0 animals were still alive, whereas after 2 weeks only 10% survived. Most importantly, 3% of L0 mice persevered through the suckling period and weaning and lived normally into adulthood. The oldest L0 animals to date are 20 months of age. No correlation was observed between the number of littermates and the survival rate of L0 mice.

LPL and HL Enzyme Activities—Fig. 2 exhibits the time course of LPL expression after Ad-LPL treatment in adult L2 mice. LPL activity measurements in PHP of adult control mice intravenously injected with 5 × 10⁸ pfu of Ad-LPL revealed that a maximum expression level was observed 7 days following virus injection. Afterward, LPL activities decreased sharply and were back to preinjection levels 3 weeks after virus application. Although it was technically not possible to determine LPL activities in PHP of newborn pups, a similar expression pattern can be assumed in Ad-LPL-treated mice during the suckling period. PCR experiments in 3–4-week-old pups demonstrated that 3 weeks after injection, no adenoviral DNA was detectable in liver and other tissues of Ad-LPL-treated mice.

Expectedly, LPL activity in PHP of adult L0 animals (12–14 weeks of age) was undetectable, whereas control animals exhibited normal LPL activity. In contrast, HL activity was increased in L0 mice by 1.7-fold when compared with L2 animals (Table I). In preheparin plasma the activities of HL were identical to those found in PHP, whereas LPL activity was not detectable (not shown).

Plasma Lipids and Lipoproteins—Plasma TG and TC concentrations were determined in L0 and L2 mice at the age of 7 days, 14 days, and 12 weeks (Table II). During the suckling period, plasma TG levels in L0 mice increased gradually and were 2.9- and 68-fold increased in 7- and 14-day-old animals, respectively, compared with L2 mice. After weaning, the hypertriglyceridemia became less severe; however, in 12-week-old animals TG levels of L0 mice were still 44-fold (fed state) and 30-fold (fasted state) higher than in controls. A similar time course was observed for the plasma TC concentrations, although the differences among the mouse genotypes were less pronounced. A moderate 1.9-fold increase in 7-day-old animals was followed by a 14.3-fold increase in 14-day-old mice. At the age of 12 weeks, TC levels of L0 animals were 4.1-fold higher in the fed state and 2.5-fold higher in the fasted state than in L2 mice.

To investigate the lipid distribution among lipoprotein subclasses, fasted plasma samples of adult L0 and L2 mice were subjected to FPLC analysis (Fig. 4). TC measurements in FPLC subfractions revealed a 137-fold increase of the chylomicron-VLDL fraction in L0 mice. In contrast, HDL-C was undetectable in L0 mice, whereas in L2 mice the majority of the plasma cholesterol content was found in the HDL fraction. To independently validate the virtual absence of HDL in L0 mice, HDL-C plasma concentrations were also measured in plasma after precipitation of β-lipoproteins. In contrast to normal HDL-C levels found in L2 animals (84.05 ± 5.3 mg/dl), L0 mice essentially lacked HDL-C (0.9 ± 0.3 mg/dl).

Analysis of ApoA-I-containing Lipoproteins by Nondenaturing Two-dimensional Electrophoresis—Two-dimensional polyacrylamide gradient gel electrophoresis of plasma samples...
from L0 and L2 mice and subsequent immunoblotting using a specific anti-apoA-I antibody identified two apoA-I-containing HDL subclasses in the plasma of L2 animals, one quantitatively major spot with electrophoretic α-mobility (α-LpA-I) and one quantitatively minor with electrophoretic pre-β-mobility (pre-β1-LpA-I) (Fig. 5). In contrast, plasma samples of L0 mice contained only traces of apoA-I in the HDL region of the gel, indicating not only the virtual absence of α-migrating mature HDL but also a drastic reduction of their precursor preβ1-LpA-I.

Analysis of ApoA-I mRNA in the Liver—Northern blotting experiments were performed to analyze apoA-I mRNA levels in the liver of L0 and L2 mice. ApoA-I mRNA concentrations were identical in rescued L0 mice compared with L2 mice (Fig. 6). Accordingly, the absence of HDL particles in the plasma of L0 mice is not a result of decreased apoA-I gene expression.

Western Blotting Analysis of ApoA-I and ApoA-II in Total Plasma and Lipoprotein Fractions—ApoA-I protein expression was determined by Western blotting analysis of total plasma, the chylomicron fraction, and the HDL fraction of L0 and L2 animals. As shown in Fig. 7, apoA-I protein was clearly detectable in total plasma of both mouse lines, and the specific signals for apoA-I exhibited similar intensities, suggesting comparable apoA-I concentrations. However, whereas in control (L2) mice essentially all apoA-I was associated with the HDL fraction, L0 mice lacked apoA-I in the HDL density region. In these mice, apoA-I was predominantly found in the chylomicron fraction. Identical results were obtained for the distribution of apoA-II (not shown). These findings indicated that in the absence of LPL-mediated lipolysis, HDL particles cannot be formed, and apoA-I of hepatic origin is retained within TG-rich lipoproteins.

To investigate renal clearance of apoA-I-containing particles, Western blotting analyses were performed with concentrated urine of L0 and L2 mice (data not shown). ApoA-I excretion was very low in both mouse lines, arguing against increased apoA-I clearance as a cause of HDL deficiency in L0 mice.

To investigate apoA-I turnover in L2 and L0 mice, the disappearance of radioactivity was followed over 6 h after injection of a single dose of iodinated mouse apoA-I. The decay curves were very similar in both mouse lines (Fig. 8), and the disappearance rates were essentially identical despite the fact that apoA-I was transported on different lipoprotein particles, namely HDL in L2 mice and chylomicrons in L0 mice.

**Plasma FFA, Ketone Bodies, and Glucose**—To investigate the effects of LPL deficiency on energy metabolism, plasma levels of FFA, ketone bodies, and glucose (Table III) were determined in fed animals during the suckling period (1 and 2 weeks of age) and in fasted adult mice (12 weeks of age). FFA concentrations in plasma of L0 mice were increased in both 2-week-old (3.7-fold) and 12-week-old (3.5-fold) animals. At 7 days of age, L0 mice also exhibited increased FFA plasma levels; however, this difference did not reach statistical significance because of the low number of available L0 mice.

The analysis of ketone bodies in plasma revealed increased concentrations in L0 mice at the age of 7 days (2-fold), 14 days (1.6-fold), and 12 weeks (1.6-fold), suggesting that the increased FFA mobilization in L0 mice led to increased ketone body production in the liver. During suckling, plasma glucose levels were slightly increased with a statistically significant difference at 2 weeks of age (+34%). However, this difference was not seen in fasted adult L0 mice.

**DISCUSSION**

Mice that lack enzymatically active LPL die prematurely at the beginning of the suckling period. This phenotype was observed in LPL knock-out mice that lack LPL protein (24), as well as in mice carrying the cld/cld mutation (25). This genetic defect in a presently unidentified locus on chromosome 17 causes the cellular retention of an incompletely processed LPL protein and the absence of active LPL in the vascular system.
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Blood was taken in the morning from 1-week-old, 2-week-old, and 12-week-old male animals of each group having free access to food (fed samples) or after 10 h of nighttime fasting (fasted samples). All values represent means ± S.D. *, p < 0.05; **, p ≤ 0.01; *** p ≤ 0.001 compared with the controls.

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>n</th>
<th>TG</th>
<th>TC</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>mg/dl</td>
<td></td>
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<tr>
<td>1 week Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L0</td>
<td>3</td>
<td>599 ± 132**</td>
<td>121 ± 12</td>
<td></td>
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<tr>
<td>L2</td>
<td>14</td>
<td>208 ± 79</td>
<td>63 ± 17</td>
<td></td>
</tr>
<tr>
<td>2 weeks Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L0</td>
<td>3</td>
<td>9453 ± 821**</td>
<td>828 ± 135*</td>
<td></td>
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<tr>
<td>L2</td>
<td>7</td>
<td>139 ± 42</td>
<td>58 ± 8</td>
<td></td>
</tr>
<tr>
<td>12 weeks Fed</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L0</td>
<td>7</td>
<td>4997 ± 1102***</td>
<td>396 ± 90**</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>5</td>
<td>113 ± 19</td>
<td>97 ± 15</td>
<td></td>
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<tr>
<td>Fasted L0</td>
<td></td>
<td>2007 ± 375***</td>
<td>172 ± 33***</td>
<td></td>
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<tr>
<td>L2</td>
<td>5</td>
<td>66 ± 7</td>
<td>69 ± 9</td>
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</table>

In contrast to mice, LPL deficiency in humans (12), cats (41), and minks (42) is not lethal. This species-specific difference and the actual cause of death in LPL-deficient mice have not been elucidated. Several hypotheses have been proposed: (i) the hypertriglyceridemia following sucking might cause respiratory dysfunction in the mouse because of the higher fat content in mouse milk (10% versus 4.5% in human milk) or differences in the anatomy of the lung capillary system; (ii) the abnormally low glucose levels in newborn L0 pups due to the low carbohydrate content in mouse milk (15% versus 30% in human milk) might cause lethal hypoglycemia; (iii) the absence of HDL particles might be incompatible with survival, because the majority of plasma lipids are transported in the HDL fraction in mice; (iv) an unidentified, possibly nonenzymatic function of LPL might be essential in mice but is not required in other LPL-deficient species.

Previous studies in which transgenic LPL expression in skeletal muscle, cardiac muscle, or liver (26–29) was achieved in otherwise LPL-deficient mice revealed that these mice can be rescued independently of the site of LPL expression. This suggested that, for survival, the organ in which LPL is expressed is irrelevant as long as sufficient amounts of active enzyme are present in the vascular system. The expression of an enzymatically inactive protein on an L0 background is not sufficient for survival (43). To investigate whether the presence of LPL was obligatory during suckling but dispensable after weaning, the transient expression of LPL by adenovirus-mediated gene transfer was utilized. Ad-LPL was injected into all animals of a litter immediately after birth. LPL expression reached a peak 7 days after injection. Subsequently, enzyme expression declined and was undetectable in weaned animals at 4 weeks of age. A similar expression pattern was observed when LPL-expressing recombinant adenovirus was administered to heterozygous LPL +/- animals (44). The transient expression of LPL after a single virus application resulted in a profound extension of viability in all L0 animals. However, only a small percentage (3%) survived the entire suckling period. These animals were growth-retarded and severely hyperlipidemic. Additionally, they exhibited marked changes in plasma FFA and ketone body concentrations.

Growth retardation was most evident during the suckling period. The effects of complete or partial LPL deficiency during suckling on body weight and development in other species have not been sufficiently studied to date. However, similar trends of decreased body weight have been reported in LPL-deficient cats and minks (41, 42). To our knowledge, data for newborn humans affected with type I hyperlipoproteinemia are not available. Apparently, the decreased availability of TG-derived FFA in sucking L0 mice is not adequately replaced by other substrates in muscle and AT, which might lead to the observed defects in body development. After weaning, when fed a chow diet with 2.5% fat, the animals recovered rapidly and exhibited similar body weight and body composition at 3–4 months of age compared with control mice. Thus, the complete absence of LPL in adult L0 animals did not affect growth. Similar results were obtained in a study of human adults affected with type I hyperlipoproteinemia. These patients were found to have normal
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**Fig. 6. ApoA-I mRNA levels in the liver of Ad-LPL-treated L0 and L2 mice.** Total RNA was isolated from liver tissues and subjected to Northern blot analysis. To detect the mouse apoA-I mRNA, a 665-base pair PCR product specific for mouse apoA-I was used as a probe.

**Fig. 7. ApoA-I levels in plasma and lipoprotein fractions.** For Western blotting analysis samples were mixed 1:1 with loading buffer (5% SDS), incubated for 10 min at 95 °C, and fractionated by SDS-polyacrylamide gel electrophoresis. Bands were visualized by an enhanced chemiluminescence assay after incubation with rabbit anti-mouse apoA-I antibody. As a second antibody, horseradish peroxidase-labeled mouse anti-rabbit antiserum was used.

AT and body weight (45). Additional evidence for normal fat mass development in the absence of LPL in AT was obtained from induced mutant mouse lines that expressed LPL exclusively in muscle but lacked the enzyme in AT (27). These animals had normal body weight and AT mass. However, their AT exhibited a profound change in fat composition. Essential fatty acids were drastically decreased and replaced by saturated and monounsaturated fatty acids in the AT lipid moiety.

Weaned animals on a chow diet (2.5% fat) live for over a year. However, in the absence of LPL the animals are severely hypertriglyceridemic. Plasma cholesterol levels are also increased as a consequence of the drastic increase in the cholesterol content in the TG-rich lipoprotein fraction. In contrast, low density lipoprotein-C and HDL-C were essentially absent in L0 mice. The absence of HDL-C is particularly remarkable, since in normal mice more than 75% of the plasma cholesterol moiety is found in HDL. HDL-C concentrations are also drastically reduced in human LPL deficiency (80–95%), although there is considerable variation. This heterogeneity is partially due to the large number of different mutations in the LPL gene, some of which cause partial absence of LPL activity in PHP, while others result in complete loss of activity (12). Similarly, both of the other existing animal models for familial chylomicronemia syndrome, namely cats and minks, were shown to produce small amounts of active LPL. In contrast to the complete LPL-deficiency in L0 mice, the residual level of LPL activity in these models might explain the essentially normal HDL-C levels (41, 42).

Low HDL levels in LPL deficiency have been attributed to the reduced formation of HDL precursors, the disturbed maturation of these precursors by acquisition of surface remnants (20), the enhanced CETP-mediated transfer of cholesteryl esters from HDL onto TG-rich lipoproteins (46), and the lack of FFA (47). In our study, the absence of CETP in mouse plasma and the increased levels of FFA (Table III) rule out the latter two explanations. The analysis of apoA-I-containing HDL in mouse plasma in two-dimensional electrophoresis revealed that both preβ1-LpA-I and α-LpA-I are absent from LPL-deficient mice despite normal apoA-I mRNA levels in the liver and normal apoA-I protein levels in plasma. ApoA-I in L0 mice, however, was predominantly associated with the chylomicron fraction. Current evidence from liver perfusion studies and experiments with isolated hepatocytes suggests that either free apoA-I (48) or poorly lipidated apoA-I (‘nascent’ HDL) (49, 50) is secreted by the liver and is subsequently remodeled by PLTP (22) and lecithin:cholesterol acyltransferase (51). Our observations indicate that these HDL precursor components fuse with TG-rich lipoproteins before they are converted to more mature preβ1-LpA-I or α-LpA-I particles. An alternative explanation for the lack of HDL, namely that mature HDL particles are first formed normally in LPL knock-out mice but subsequently are absorbed by TG-rich lipoproteins, is unlikely for the following reasons. First, other hypertriglyceridemia models in the mouse that result in high plasma TG levels and have either low or normal LPL expression exhibited reduced HDL levels, but the essential disappearance of HDL, which would be expected if mature HDL would simply be absorbed by TG-rich lipoproteins, was not observed (43, 52). Second, HDL precursor particles (preβ1-LpA-I) should be detected in the plasma of LPL knock-out mice if only mature HDL particles (α-LpA-I) were absorbed by TG-rich lipoproteins. However, careful analysis by two-dimensional polyacrylamide gradient gel electrophoresis revealed that preβ1-LpA-I particles are barely detectable in the plasma of LPL knock-out mice. This finding suggests that either these particles fuse with TG-rich lipoproteins before they

**Table III**

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>n</th>
<th>Glucose (mg/dl)</th>
<th>Ketone bodies (mg/dl)</th>
<th>FFA (mg/dl)</th>
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<tbody>
<tr>
<td>1 week</td>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L0</td>
<td>3</td>
<td>129.3 ± 18</td>
<td>18.4 ± 4.6*</td>
<td>34 ± 9</td>
<td></td>
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<tr>
<td>L2</td>
<td>14</td>
<td>102.2 ± 14</td>
<td>9 ± 2.4</td>
<td>21 ± 6</td>
<td></td>
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<tr>
<td>2 weeks</td>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L0</td>
<td>3</td>
<td>161.6 ± 19.7*</td>
<td>17.4 ± 4.1</td>
<td>63.2 ± 9.2**</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>7</td>
<td>120.5 ± 11.7</td>
<td>10.3 ± 3.2</td>
<td>17 ± 3.2</td>
<td></td>
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<tr>
<td>12 weeks</td>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L0</td>
<td>5</td>
<td>119.8 ± 8</td>
<td>13.3 ± 1.2**</td>
<td>76 ± 11.3**</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>6</td>
<td>122.6 ± 5</td>
<td>8.4 ± 0.8</td>
<td>22 ± 6.6</td>
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**Fig. 8. ApoA-I turnover.** ApoA-I was isolated from mouse plasma, labeled with 125I, and injected into the tail vein. The percentage of radioactivity remaining in plasma was determined at the times indicated.
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accumulate to detectable levels or the precursor of these particles (apoA-I) has already been absorbed, thus preventing pre-β-LpA-I formation. Precedence for the accumulation of HDL precursor particles but not mature HDL was reported in a recent publication describing an ABC-A1-deficient mouse model (53). This study demonstrated, using two-dimensional polyacrylamide gel electrophoresis, that in ABC-A1-deficient mice pre-β-LpA-I particles are formed normally, yet mature HDL particles (α-LpA-I) are absent. In LPL-deficient mice, not even these HDL precursor particles accumulate detectably, which argues for a defect in an early step of HDL maturation in these mice. Presumably, that step is the dissociation of apoA-I-containing surface remnants as a result of LPL-mediated hydrolysis of the TG core of TG-rich lipoproteins. The importance of surface remnants and other lipolysis products for the maturation of HDL was previously shown in vitro experiments (47, 54, 56) and PLTP knock-out mice, which are defective in the transfer of phospholipids from VLDL to HDL and which also show marked decreases in the serum levels of HDL-C, apoA-I, preβ-LpA-I, and α-LpA-I (22).

Increased clearance of apoA-I into the extravasal space (57, 58) was not observed in L0 mice, because apoA-I concentrations were very low in the urine of both LPL-deficient and control mice. Additionally, the disappearance rates of apoA-I from plasma were identical in L2 and L0 mice, indicating similar turnover kinetics despite the fact that apoA-I was transported in HDL in control mice and in chylomicrons in L0 mice. Taken together, our results support the following conclusions. HDL components are produced normally but are rapidly absorbed by TG-rich lipoproteins at an early stage of HDL maturation before pre-β-LpA-I particles are formed. This results in an essential absence of HDL precursor particles, absence of mature HDL, and absence of HDL cholesterol in LPL-deficient mice. These observations are important because recently it has become widely accepted that the major factors responsible for HDL biogenesis are apoA-I expression by the liver, the subsequent cholesterol absorption from peripheral tissues facilitated by ABC-A1, and finally particle remodeling mediated by lecithin:cholesterol acyltransferase, CETP, hepatic lipase, and PLTP (15–19, 21). We show that these processes, at least in mice (which lack CETP), are not sufficient for normal HDL maturation and HDL cholesterol levels when LPL is not present. Thus, LPL, together with apoA-I and ABC-A1, is required for the formation of mature HDL particles.

Recently, the partial absence of LPL in heterozygous LPL knock-out mice has been shown to affect insulin secretion from β-islets, leading to decreased plasma glucose levels in these animals (59). Accordingly, the complete absence of LPL in heterozygous patients with type I hyperlipoproteinemia, glucose tolerance and blood glucose were normal in a large number of homozygous patients with LPL deficiency when compared with normal subjects (60, 61).

Interestingly, FFA and ketone body concentrations were increased in reared L0 mice. This observation was made in suckling animals as well as in adult mice and has not been reported for other LPL-deficient species. The mechanism behind this observation has yet to be elucidated; however, one could speculate that the observed increase in HL activity might be responsible for the increased FFA concentrations. Alternatively, it is conceivable that inadequate incorporation of FFA into adipose tissue and muscle of L0 mice might contribute to the plasma FFA pool. This phenotype was recently observed in mice expressing human LPL defective in heparin binding (55).

Increased FFA transport to the liver combined with decreased VLDL synthesis as a result of the presence of large amounts of TG-rich lipoproteins in plasma could cause hepatic lipid accumulation. This hypothesis is consistent with increased hepatic lipid levels and hepatomegaly commonly observed in LPL-deficient humans and mice (12, 24). Accordingly, the increased availability of FFA in the liver might result in increased β-oxidation rates and increased ketone body formation in L0 mice.

In conclusion, the life span of LPL-deficient mice was prolonged by adenosvirus-mediated gene therapy during the suckling period. A small fraction of these animals survived the entire suckling period and developed into normal adulthood with a life expectancy of at least 20 months when kept on a low fat diet. The absence of HDL-C and HDL-apoA-I suggested an essential role for LPL in the maturation of HDL. Regarding energy metabolism, the complete absence of LPL in all tissues including β-islets in reared, adult animals had no effect on plasma glucose and insulin levels; however, FFA and ketone body concentrations were increased. Adult LPL-deficient mice will be extremely useful for the investigation of the role of LPL and LPL mutants in lipid, lipoprotein, and energy metabolism.

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LIPIDS AND LIPOPROTEINS: Adenovirus-mediated Rescue of Lipoprotein Lipase-deficient Mice: LIPOLYSIS OF TRIGLYCERIDE-RICH LIPOPROTEINS IS ESSENTIAL FOR HIGH DENSITY LIPOPROTEIN MATURATION IN MICE

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