Induced Mutant Mice Expressing Lipoprotein Lipase Exclusively in Muscle Have Subnormal Triglycerides yet Reduced High Density Lipoprotein Cholesterol Levels in Plasma*

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To determine the contribution of muscle lipoprotein lipase (LPL) to lipoprotein metabolism, induced mutant mice were generated that express human LPL exclusively in muscle. By cross-breeding heterozygous LPL knockout mice with transgenic mice expressing human LPL only in muscle, animals were obtained that express human LPL primarily in skeletal muscle on either the null (L0-MCK) or normal (L2-MCK) LPL backgrounds, and these were compared with control littersmates (L2). Fed and fasted post-heparin plasma (PHP) LPL activities were increased 1.4- and 2.3-fold, respectively, in L2-MCK mice and were normal in L0-MCK mice compared with controls. The specific enzyme activities of human LPL in mouse plasma was comparable to human LPL in human PHP. Skeletal muscle LPL activity was increased in both L2-MCK and L0-MCK mice in the fed (6.6-fold) and fasted (4.2-fold in L2-MCK; and 3.4-fold in L0-MCK) states. Adipose tissue LPL mRNA and activity were not detectable in L0-MCK mice. Growth and body mass composition were similar among all groups. In the fasted and fed state, L2-MCK mice had 31% and 53% reductions, respectively, in plasma triglycerides (TG), compatible with increased PHP LPL activity. Unexpectedly, both in the fasted and fed state the L0-MCK mice also had reduced TG (22%), despite normal PHP LPL activities. Very low density lipoprotein (VLDL) turnover studies revealed that the decreased TG were due to increased particle fractional catabolic rate in both L2-MCK and L0-MCK mice. Despite reduced TG, both L2-MCK and L0-MCK mice showed reduced high density lipoprotein (HDL) cholesterol levels (16% and 19%, respectively). HDL turnover studies indicated increased HDL cholesterol ester fractional catabolic rate in the L2-MCK and L0-MCK compared with control mice. In summary, these studies suggest that muscle LPL is particularly potent with regard to VLDL metabolism and is sufficient to compensate for the lack of LPL in other tissues with regard to lipolyzing VLDL particles. With regard to HDL, muscle LPL expression does not result in normal levels due to enhanced breakdown either by mediating accelerated HDL clearance or by failing to establish normal HDL particles that are then cleared more quickly than normal. These studies provide new insights on the tissue-specific effects of LPL on lipoprotein metabolism.

Lipoprotein lipase (LPL) plays a central role in lipoprotein metabolism. It is synthesized in most extrahepatic tissues and is transported to the capillary endothelium where it hydrolyzes triglycerides (TG) contained in circulating chylomicron and VLDL particles (for review, see Refs. 1–3). The highest levels of LPL expression are in adipose tissue (AT) and muscle, where the liberated free fatty acids (FFAs) enter either storage (4) or oxidative (5) pathways, respectively. In addition, LPL-mediated lipolysis promotes the exchange of lipids and surface apolipoproteins between lipoprotein particles, thereby affecting the size, amount, and metabolism of not only TG-rich lipoproteins but of LDL and HDL as well (6). More recently, in vitro studies have shown that LPL can bridge lipoproteins to proteoglycan matrices on cell surfaces and/or can serve as a ligand for lipoprotein receptors (7, 8, 55, 56). However, whether this occurs in vivo has yet to be determined.

The LPL protein is encoded by a single gene on chromosome 8 (9, 10), which produces a structurally similar enzyme in all tissues thus far examined. However, differences exist between tissues with regard to the regulation of LPL expression and activity, implying differences in function. For example, during fasting the enzyme is markedly down-regulated in AT but is unchanged or even induced in skeletal (SM) and cardiac (CM) muscle, whereas the opposite occurs in the fed state (11, 12). It is thought that in fasting this directs fat calories to muscle to serve as fuel and at times of feeding this promotes fat storage in adipose tissue. It has been proposed that dysregulation of this process could lead to weight loss or obesity depending on the balance between LPL expression in the muscle and AT (13–16). Based on traditional systems, there is very little information about the role of tissue-specific expression of LPL on lipoprotein metabolism.

Induced mutant mice provide an opportunity to test the role of tissue-specific expression of LPL on lipoprotein metabolism.
We have previously generated both LPL knockout (17) as well as transgenic mice overexpressing LPL specifically in both skeletal and cardiac muscle (18). Total LPL deficiency leads to lethal hypertriglyceridemia within 18 h of life, at which point knockout mice demonstrate absence of HDL cholesterol (HDL-C) as well as markedly reduced intracellular lipid stores. Mice that overexpress LPL in both SM and CM on the otherwise normal LPL background have, proportional to the level of LPL transgene expression, increases in muscle FFA concentrations, metabolic organelles (mitochondria, peroxisomes), and rate of development of myopathy. These data taken together provided strong evidence for LPL as a vital gatekeeper for the entry of FFA into tissues and, more specifically, proved that excess FFA can be successfully targeted to a given tissue via changes in LPL expression alone.

To further investigate the tissue-specific role(s) of muscle LPL in the absence of LPL in any other tissue, we have crossed the muscle-specific transgene onto the LPL knockout background, thereby producing mice that express LPL exclusively in muscle tissues (L0-MCK). Unlike LPL knockout mice, L0-MCK mice demonstrated normal growth, body mass composition, and life spans. Despite normal PHP LPL activities, cross-gene construction corresponding to the 5' transgene, primers were used that amplified the region of the LPL DNA isolated from tail tips as shown in Fig. 1. To detect the human LPL transgene expression, increases in muscle FFA concentrations, metabolic organelles (mitochondria, peroxisomes), and rate of development of myopathy. These data taken together provided strong evidence for LPL as a vital gatekeeper for the entry of FFA into tissues and, more specifically, proved that excess FFA can be successfully targeted to a given tissue via changes in LPL expression alone.

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**MATERIALS AND METHODS**

**Generation of Induced Mutant Mice—**We have previously reported both heterozygous LPL knockout mice (mL1, 129/b6 strain; Ref. 17) and LPL transgenic mice made with a human LPL minigene driven by the mouse creatine kinase (MCK) promoter (b6/CBA strain; Ref. 19). Three lines of transgenic mice were studied with low, medium, and high transgene expression. These lines were designated MCK-L, MCK-M, and MCK-H, respectively. All three lines only expressed human LPL mRNA in SM and CM, but in no other tissues. The MCK-L line was used in the current studies since these animals do not develop the myopathy seen in mice of the higher expressing MCK-M and H lines. The MCK-L line has been more descriptively renamed mL2/MCK-LPL+ to delineate that these mice carry the human transgene (MCK-hLPL) on a background of two intact mouse LPL alleles (mL2).

As shown in Table I, mL1 and mL2/MCK-hLPL+ were cross-bred to obtain LPL knockout heterozygotes which contained the muscle-specific transgene (mL1/MCK-hLPL+). mL1/MCK-hLPL+ mice were then backcrossed with mL1 mice to produce the following littersmates: mL2 mice (renamed mL2/MCK-hLPL+) containing two functional copies of the mouse LPL gene with the addition of the transgene, primers were used that amplified the region of the LPL DNA isolated from tail tips as shown in Fig. 1. To detect the human LPL transgene expression, increases in muscle FFA concentrations, metabolic organelles (mitochondria, peroxisomes), and rate of development of myopathy. These data taken together provided strong evidence for LPL as a vital gatekeeper for the entry of FFA into tissues and, more specifically, proved that excess FFA can be successfully targeted to a given tissue via changes in LPL expression alone.

**Screening of Induced Mutant Mice—**During all breeding experiments, genotypes were determined by double PCR analysis of genomic DNA isolated from tail tips as shown in Fig. 1. To detect the human LPL transgene, primers were used that amplified the region of the LPL minigene corresponding to the 5' 410 bp of the LPL cDNA (Fig. 1A). The primers used in the reaction were: I, 5' -GTGACCTGC-CAAGGGCTGACTTACAGGA-3'; II, 5'-CTCTAGGGAAGAGGCTGC-CCAC-3'. PCR conditions in a reaction volume of 50 μl were as follows: 200–300 ng of tail-tipped DNA, 250 ng of each primer, 0.02 mM dNTPs, 1 unit of DynaZyme II DNA polymerase, denaturation at 95 °C for 5 min for a 1 cycle, denaturation at 94 °C for 1 min, annealing and extension at 72 °C for 3 min for a total of 45 cycles. In both reactions PCR buffer was used containing 200 mmoles Tris-HCl (pH 8.3), 250 mM KCl, 20 mM MgCl2, and 0.1% BSA.

**RNA Analysis—**RNA was prepared, via the acid guanidium thiocyanate-phenol chloroform extraction method of Chomczynski and Sacchi (19), from SM, CM, and AT obtained from fed mice from all groups. For Northern blot analysis, 10 μg of total RNA was separated by 1% formaldehyde-agarose gel electrophoresis and subsequently blotted onto a nylon membrane (Hybond N+, Amersham). The blot was prehybridized for 6 h at 65 °C and hybridized overnight at 65 °C in a buffer containing 0.15 M sodium phosphate (pH 7.2), 1 mM EDTA, 7% SDS, and 1% BSA. To detect mouse LPL mRNA, a 1.6-kb EcoRI-Fral fragment from the mouse LPL cDNA was used as a probe. To differentiate human LPL mRNA from the endogenous mouse mRNA, a species-specific DNA-probe containing a 1-kb EcoRI fragment from exon 10 of the human LPL gene was used (18).

**Lipolytic Enzymes—**LPL activity in tissue and PHP was determined in 3-month-old male mice. Enzyme activities were determined in both the fed and the fasted states. Animals were fasted by removing the food at 8 a.m. and performing the analysis at 5 p.m. Mice were injected intraperitoneally with sodium heparin (1 unit/g body weight), and post-heparin plasma was taken after injection of epididymal fat pads, heart, and skeletal muscle specimens (100 mg each) were surgically removed from the animals and put into ice-cold tubes containing 1 ml of Dulbecco's modified Eagle's medium with 2% BSA and 2 units/ml heparin. After mincing the tissue with scissors, it was incubated in medium for 1 h at 37 °C. Enzyme activities of LPL in muscle tissue and adipose tissue as well as LPL and hepatic triglyceride lipase in PHP of mice was assayed as described earlier (18). The assay of PHP-lipolytic activities was performed in the presence or absence of 1 mM NaCl to estimate both the LPL and HL activities. LPL activity was calculated as the portion of total lipase activity inhibited by 1 μM NaCl. To differentiate between human and mouse LPL activity in post-heparin plasma and tissue extracts, the assay mixtures were preincubated for 1 h at 4 °C in the presence of or absence of 0.1 μg of monoclonal antibody 5D2 (kindly provided by Dr. J. D. Brunzell, University of Washington, Seattle, WA) (20). This antibody specifically inhibits the human but not the mouse enzyme in the LPL assay. Specific enzyme activities were calculated in milliunits/mg of LPL protein. One milliunit of enzyme activity corresponds to 1 μM FFA released in 1 min.

**LPL Mass Determinations—**Protein mass of human LPL was determined commercially available ELISA kit (MARRIT-LPL, Dainippon Pharmaceuticals Co. Ltd., Osaka, Japan). The protocol provided by the manufacturer was followed.

**Lipid and Lipoprotein Analysis—**Male mice were fed a regular mouse chow (4.5% fat) diet. Blood was taken at 8 a.m. with the animals having free access to food (fed samples) and in the evening after they had fasted 5 h during the day (fasted samples). The HDL fraction of plasma was isolated using the HDL cholesterol reagent Sigma. Total plasma cholesterol and HDL-C were determined by the CHOD-PAP method (Boehringer Mannheim). To compare several methods for HDL-C quantitation, all initial analyses were performed in duplicate and HDL-C was determined after precipitation of apolipoprotein B containing lipoproteins (VLDL + LDL) with the HDL cholesterol reagent ELSA kit (MARRIT-LPL, Dainippon Pharmaceuticals Co. Ltd., Osaka, Japan). The protocol provided by the manufacturer was followed.

**In Vivo Removal of Labeled VLDL—**To prepare the labeled VLDL, control mice were injected intravenously with [3H]palmitate (200 μCi) and bled 45 min after the injection. Plasma samples were ultracentrifuged to obtain the VLDL fraction. TLC on Silica Gel G plates using hexane-diethylether-acetic acid solution (83:16:1) as the solvent was used to perform that 85–90% of the label was in the TG fraction before the VLDL preparations were used for further studies. Radiolabeled VLDL clearance was determined in 10 animals of each line. Mice were injected intravenously with 200,000 dpm of [3H]-labeled VLDL, and the disappearance of the radioisolation labeled VLDL was determined from plasma samples drawn 2, 5, 10, 20, 40, 75, and 120 min after the injection. VLDL-TG kinetics were analyzed using a one- or two-pool...
model based on a main VLDL pool, with the assumption that the remnant pool is derived entirely from the main pool (57). FCR were determined by fitting a one- or two-pool model by minimizing a weighted sum of squares of differences between the observed data and model-generated values (the weighting assumes a constant coefficient of variation or percent error). The procedure is described in detail in Ref. 57. The program, called POOLFIT, was developed by one of the authors (R. Ramakrishnan) and has been used in a variety of tracer kinetic studies in the past (17).

Vitamin A Fat Tolerance Test—

Animals of each line were given an intragastric bolus of RE in corn oil (3000 units), followed by 100 ml of air. Mice were bled before and 1, 2, 4, and 10 h after vitamin A administration. 100 ml of plasma were placed in a 13 × 100-mm tube containing 500 μl of saline. Subsequently, 40 ng of retinyl acetate (in 200 μl of mobile phase), 500 μl of methanol, and 500 μl of mobile phase were added. The tubes contents were vortexed after each addition. Phase separation was achieved by centrifugation at 350 g for 15 min. The upper phase was removed and analyzed for retinoids. Retinol and retinyl ester quantification was essentially performed as described previously (21). The analytes were separated at a flow rate of 2 ml/min on a EXsil 100 column (5 μm, 200 × 4.6 mm), with hexane/butylchloride/acetonitrile (90.7:2.5:2.5) containing 0.01% (v/v) acetic acid as the mobile phase and fluorimetric detection (Ex = 350 nm, Em = 510 nm). Under these conditions retention times were 1.2, 1.6, and 4.1 min for retinyl palmitate, retinyl acetate, and retinol, respectively. Vitamin A clearance was assessed by comparing peak heights and total areas under the plasma decay curve of retinyl palmitate (RP).

In Vivo HDL-CE Turnover Studies—Plasma from control mice was incubated with [3H]cholesteryl oleoyl ether (specific activity 46.6 Ci/mm; Amersham), Intralipid, and human cholesteryl ester transfer protein for 16 h at 37 °C as described previously (22). The labeled HDL fraction was isolated by sequential ultracentrifugation between d1.063 and 1.21 g/ml, dialyzed against five changes of 1 liter of 0.9% NaCl and 1mM EDTA for 20 h, and used immediately. Mice were injected intravenously with labeled HDL, where the injected mass was less than 5% of the mouse HDL pool. Seventy microliters of blood was taken from the retro-orbital plexus at 10 min, 1.5, 3, 8, and 24 h after injection for determination of plasma radioactivity. The fraction catabolic rates for HDL cholesteryl linoleyl ether were calculated from the plasma decay curves, assuming a two-pool model by the Matthews method (57).

Nondenaturing Gradient Gel Electrophoresis—HDL particle size distribution was evaluated as described previously (23, 24). Plasma specimens from each mouse were subjected to gradient gel electrophoresis. In a 175-μl Airfuge tube, 40 μl of plasma was adjusted to d 1.21 g/ml with 1.35 g/ml KBr and 1 mM EDTA and centrifuged at 100,000 × g for 8 h (Airfuge). 30 μl were then aspirated from the top of the tube and
Muscle-specific LPL Expression

Table I
Breeding strategy to obtain transgenic mouse lines that lack the endogenous LPL gene

| Mating 1: | Progeny: |
|----------|----------|
| mL1 × mL2/MCK-hLPL | mL1, mL2, mL2/MCK-hLPL |
| Mating 2: | Progeny: |
| mL1/MCK-hLPL × mL1 | mL1, mL2/MCK-hLPL (L2-MCK), mL1/MCK-hLPL, mL0/MCK-hLPL (L0-MCK) |

* mL2, wild type; mL1, heterozygous LPL-null; mL0, homozygous LPL-null; MCK-hLPL, muscle-specific human LPL minigene driven by the MCK promoter.

RESULTS

Generation of Mice Expressing LPL Exclusively in Muscle—To investigate the metabolic consequences of tissue-specific expression of LPL exclusively in muscle, transgenic mouse lines were generated that expressed the enzyme in SM and CM only and not in any other tissue. As shown in Table I, two crosses were necessary to produce littermates that either were controls (L2) or expressed human LPL in muscle on either the normal (L2-MCK) or LPL-null (L0-MCK) backgrounds. All animals had similar growth curves and body mass composition.

LPL gene expression was assessed by mRNA and enzyme activity in SM, CM, and AT. To differentiate between mouse and human LPL mRNA, species-specific probes were used as described previously (18). Northern blot analysis (Fig. 2) revealed that mouse LPL mRNA of the expected size (Fig. 2A, 3.8 kb) was detected in all tissues from L2 and L2-MCK mice, while no signal was observed in any tissue from L0-MCK animals. When a human LPL-specific probe was hybridized, a signal of the expected size (Fig. 2B, 3.6 kb) was found in both CM and SM of L2-MCK and L0-MCK mice but not in L2 mice, indicating the successful tissue-specific expression of the human transgene in L2-MCK and L0-MCK mice.

During fasting, LPL activities were increased 4.2- and 3.4-fold in L2-MCK and L0-MCK mice, respectively. CM activity was increased 1.4-fold in L2-MCK mice in both fed and fasted state, while decreased 82% and 64%, respectively, in fed and fasted L0-MCK mice. AT LPL activities were elevated 1.8-fold in the fed state and unchanged in the fasted state in L2-MCK mice when compared with controls, while undetectable in L0-MCK mice.

PHP LPL activities were measured in the fed and fasted states. As shown in Table II, fasted PHP LPL activities were increased 2.3-fold in L2-MCK mice and 1.3-fold in L0-MCK mice when compared with L2 mice. Fed PHP LPL activities were increased 1.4-fold in L2-MCK mice, while L0-MCK mice displayed activities similar to those of L2 mice. Fed and fasted PHP hepatic triglyceride lipase activities were increased 26% and 36%, respectively, in L2-MCK mice when compared with controls, while slightly decreased in L0-MCK mice.

In addition to LPL enzyme activities, the protein concentration of human LPL was also determined in PHP and extracts of SM in L2-MCK and L0-MCK mice. Table III shows that both L2-MCK and L0-MCK animals express similar amounts of human LPL in PHP and SM extracts. Human LPL in L2 animals was undetectable, proving the specificity of the ELISA for human LPL. Increased LPL activities due to transgene
expression correlated well with LPL mass in PHP. The specific activities of human LPL in the plasma of L0-MCK mice were not significantly different compared with human LPL in human PHP, indicating that LPL expression from the human transgene produced an enzyme with normal enzymatic properties. No indication was found for the production of excess inactive LPL in SM of L2-MCK or L0-MCK mice.

Plasma Lipids and Lipoproteins—The effects of muscle-specific expression of LPL on plasma lipids were investigated in both the fed and fasted states and results are shown in Table IV. When fasted, TG levels of chow-fed L2-MCK and L0-MCK mice decreased 31% and 22%, respectively. These differences were more pronounced in the fed state, where L2-MCK and L0-MCK mice showed 53% and 22% reductions in TG, respectively, when compared with L2 mice. Fed L2-MCK and L0-MCK mice showed significant decreases in total plasma cholesterol (TC) concentrations (14% and 20%, respectively) when compared with controls, while fasted animals showed similar, yet non-significant trends. Likewise, fed HDL-C levels were decreased 17% and 19% in L2-MCK and L0-MCK mice, respectively, accounting for the majority of the reduction in TC. The decrease of HDL-C was observed independent of the method used for HDL-C quantitation including precipitation of apolipoprotein B containing lipoproteins, HDL separation by HPGC, or HDL isolation by preparative ultracentrifugation (data not shown). In both the fed and fasted states, plasma FFA were reduced in both L2-MCK (–58%) and L0-MCK (–33%) mice as compared with L2 controls.

Clearance of Triglyceride-rich Lipoproteins—To determine the metabolic cause of the reduced TG seen in both L2-MCK and L0-MCK mice, turnover experiments were performed. Clearance rates of VLDL were measured in male mice from each group. LPL mass was measured using a commercial Markit-F LPL ELISA kit from Dainippon Pharmaceuticals Co. (Osaka, Japan). ND, not determined.

A vitamin A fat tolerance test was used to assess dietary fat (chylomicron) clearance in both L2-MCK and L0-MCK mice. Since hydrolysis of chylomicrons by LPL is the first step in dietary fat clearance, this test is an appropriate way to assess the adequacy of the enzyme. In the absence of malabsorption, the level of plasma RP over time is inversely proportional to the rate at which chylomicrons are cleared from the circulation. Fig. 4B shows that plasma RP levels peaked at 2 h and the peak height was decreased by 47% (p < 0.01) and 17% (p < 0.05) in

**TABLE II**

Post-heparin plasma lipolytic activities

| Mice       | n | LPL activity (μM FFA/g/h) | HL (μFFA/ml/h) |
|------------|---|--------------------------|----------------|
| Fasted     |   |                          |                |
| L2         | 10 | 10.4 ± 4.2               | 2.5 ± 0.8      |
| L2-MCK     | 8  | 24.3 ± 4.2**             | 3.4 ± 0.5**    |
| L0-MCK     | 4  | 13.9 ± 1.8               | 1.8 ± 0.2      |
| Fed        |   |                          |                |
| L2         | 9  | 11.9 ± 1.7               | 2.3 ± 0.3      |
| L2-MCK     | 8  | 16.5 ± 3.0**             | 2.9 ± 0.5*     |
| L0-MCK     | 10 | 10.2 ± 2.6              | 1.9 ± 0.3*     |

**TABLE III**

LPL mass and specific activities of human LPL in human PHP, mouse PHP, and mouse skeletal muscle of L0-MCK mice

| n | LPL mass [ng/ml] | Specific activity [milliunits/ng] |
|---|-----------------|---------------------------------|
| Human PHP | 4 | 255 ± 62 | 0.39 ± 0.08 |
| L2-MCK PHP | 5 | 443 ± 47 | ND |
| L0-MCK PHP | 6 | 433 ± 98 | 0.32 ± 0.10 |
| L0-MCK SM | 6 | 349 ± 114 | 0.22 ± 0.10 |

**FIG. 3** Fed (A) and fasted (B) tissue LPL activities. The epididymal fat pads, heart, and skeletal muscle specimens (100 mg each) were incubated in 1 ml of Dulbecco’s modified Eagle’s medium with 2% BSA and 2 units/ml heparin for 1 h at 37 °C. The activity of LPL was assayed as described earlier (18). All values represent means ± S.D. Age of male animals at the time of analysis: 8–12 weeks.
TABLE IV

Plasma lipid, lipoprotein, and FFA concentrations in transgenic and control mice on chow diet

Blood was taken in the morning from male animals having free access to food (fed) or in the evening after they had fasted 8 h during the day (fasted). The concentrations of TG, TC and FFA were measured enzymatically. All values represent means ± SD. Age of animals at the time of analysis: 8–12 weeks. *, p < 0.05; **, p ≤ 0.01 compared to the controls.

| n | Lipid analysis of mice on chow diet |
|---|-----------------------------------|
|   | TG (mg/dl) | TC (mg/dl) | HDL-C (mg/dl) | FFA (mM/liter) |
| Fasted |       |         |              |               |
| L2    | 8 ± 3   | 75 ± 13  | 61 ± 12      | 2.1 ± 0.2     |
| L2-MCK | 5 ± 3** | 70 ± 4   | 54 ± 8       | 1.0 ± 0.2**   |
| L0-MCK | 5 ± 3   | 67 ± 10  | 54 ± 9       | 1.1 ± 0.2**   |
| Fed   | 24 ± 10 | 100 ± 19 | 84 ± 22      | 1.2 ± 0.2     |
| L2-MCK | 19 ± 5**| 86 ± 16* | 70 ± 16*     | 0.5 ± 0.2***  |
| L0-MCK | 22 ± 5* | 80 ± 18**| 68 ± 20**    | 0.8 ± 0.4     |

L2-MCK and L0-MCK mice, respectively. The area below the RP disappearance curves was decreased 47% (p < 0.05) in L2-MCK animals and 19% in L0-MCK animals, although this difference was not statistically significant and has to be considered as a trend. Thus, there was an accelerated clearance of chylomicrons as well as VLDL in both L2-MCK and L0-MCK mice.

HDL Metabolism—HDL turnover experiments were performed to determine the metabolic cause of the reduced HDL-C seen in both L2-MCK and L0-MCK mice. Male mice from each group were injected with mouse HDL, which had been isolated and core-labeled with [3H]cholesterol oleyl ether. As shown in Fig. 5A, the disappearance of radioactivity was accelerated in both L2-MCK and more so in L0-MCK when compared with controls. HDL-CE FCR were calculated using the Matthews method (57) and transport rates were estimated as FCR multiplied by HDL-CE pool size. The results are summarized in Table VI. L0-MCK mice demonstrated a highly significant 18% reduction in HDL-CE, which was associated with a 17% increase in HDL-CE FCR. In L2-MCK animals a similar, although less pronounced decrease of HDL-CE (-10%) and increase of HDL-CE FCR (+9%) was observed, which became statistically significant only if two outliers were excluded from the statistical analysis. HDL particle size was measured by gradient gel electrophoresis on individual samples of HDL (d 1.063–1.21 g/ml) from each animal and was found to be reduced in L2-MCK and even more so in L0-MCK mice when compared with controls (Fig. 5B). Average HDL particle sizes were 5.34, 5.28, and 5.15 nm in L2, L2-MCK, and L0-MCK mice, respectively. Although this trend toward smaller HDL particles in both L2-MCK and L0-MCK mice was observed in several experiments, the differences did not reach statistical significance.

DISCUSSION

The goal of the current study was to investigate the role of muscle LPL in lipoprotein metabolism. The strategy we chose was to breed a human LPL transgene that expressed exclusively in SM and CM on to both a normal mouse LPL background (L2-MCK) and a background in which the mouse LPL gene had been knocked out (L0-MCK). The strategy for creating these mice was complicated by two conditions. Since the homozygous LPL knockout condition is a neonatal lethal, it was necessary to introduce the human LPL transgene onto the heterozygous LPL knockout background. Moreover, since the knockouts and the transgenics were both outbreds, it was necessary to devise a breeding scheme that would produce littermates with the relevant genotypes (L2 (control), L2-MCK, and L0-MCK) for comparative studies. Both of these problems were surmounted by cross-breeding heterozygous LPL knockout mice (17) with heterozygotes that also contained a human LPL transgene driven by the mouse creatine kinase promoter, previously shown to only express human LPL in SM and CM (18). L2-MCK and L0-MCK mice had normal growth and body mass composition, but had mild to marked perturbations in the metabolism of both TG-rich and HDL lipoproteins.

One concern at the outset of these studies was whether L0-MCK mice that only express LPL in SM and CM would have some global disturbance in metabolism resulting in nonspecific effects on the lipoprotein system. The observation that these mice had normal growth and body mass composition, but had mild to marked perturbations in the metabolism of both TG-rich and HDL lipoproteins.
time of their death at 18 h of age have failed to develop adipose tissue, as occurs in control mice. Therefore, although humans with LPL deficiency survive to adulthood and have adipose tissue (29, 30), in the mouse one might have expected a postnatal role for AT LPL in the formation of adipose tissue and perhaps additional roles for LPL expressed in other tissues. The observation that the L0-MCK mice appear normal enhances the probability that the findings reported here are relevant to muscle-specific LPL effects on lipoproteins and not the consequence of more global changes in these mice. AT LPL is thought to play a major role in AT uptake of FFAs prior to storage, and the findings in the L0-MCK mice suggest that alternate mechanisms for fat storage exist, which are capable of replacing AT LPL.

The tissue beds with the two largest contributions to total body LPL are muscle and AT, with AT thought to contribute greater than 50%. The observation that the L0-MCK mice had significantly reduced TG demonstrates that SM and CM expression of LPL could meet the body’s need for lipolysis of TG-rich lipoproteins and that expression in AT is not necessary. In addition, since the L0-MCK mice had normal postheparin plasma LPL activity and reduced TG levels, the data suggest a particularly potent effect of SM LPL and CM LPL compared with AT LPL. Although the current study is the first to test the relative effects of muscle and AT LPL on plasma TG levels directly, in agreement are several studies in humans, which have shown better correlations between plasma TG levels and SM LPL compared with AT or post-heparin plasma LPL activities (31-37). In addition, the TG lowering effect of exercise or fibrates (clofibrate and gemfibrozil) in humans and rats correlated best with the induction of LPL activity in SM and/or CM, but not AT (38-41). Thus, it appears that the major determinant of plasma TG is muscle LPL.

Despite increased LPL-mediated TG lipolysis in SM of L2-MCK and L0-MCK mice resulting in decreased plasma triglycerides, plasma FFA levels were found to be decreased. This suggested that most of the FFA generated by SM-LPL are absorbed by the underlying tissue bed and do not enter the plasma pool. This observation is consistent with our earlier report showing low plasma FFA levels and unchanged plasma ketone body concentrations in animals with as high as a 24-fold overexpression of LPL in SM (18). LPL deficiency in AT and the resulting decrease of FFA delivery to AT might have an inhibitory effect on the lipolysis of AT fat by hormone-sensitive lipase. Although speculative, this compensatory mechanism might additionally contribute to low plasma FFA levels.

VLDL turnover and vitamin A fat tolerance tests were used to show that muscle LPL expression decreases plasma TG levels by increasing the catabolism of both hepatic and intestinally derived TG-rich lipoproteins, respectively. This is presumably mainly by lipolysis of the TG contained in these lipoprotein particles. However, we cannot exclude the possibility that severalfold overexpression of LPL in SM might also increase receptor-dependent or independent uptake of whole lipoprotein particles due to LPL-mediated bridging. In vitro studies have shown that the addition of LPL to a variety of cell types, regardless of the enzyme being enzymatically active or inactive, leads to increased uptake of whole VLDL particles (7, 8). It is of interest that a VLDL receptor is generally expressed in tissues known to produce the highest amounts of LPL, namely muscle and AT (42, 43). Similar specific activities of human LPL in L0-MCK mice and human PHP exclude the production of large amounts of inactive LPL in our transgenic animals, which might affect whole particle uptake prior to lipolysis. However, we can at this point not exclude the possibility that increased synthesis of normally active LPL in SM might in addition to increased TG hydrolysis also cause increased receptor-mediated lipoprotein uptake. Regardless of the specific mechanism, these studies indicate that SM LPL shows a striking correlation with, and may therefore be the major determinant of, plasma TG.

In the fed state both L2-MCK and L0-MCK mice have significantly reduced HDL-C levels. This occurred in the presence of increased SM and CM LPL activities and decreased TG levels. Due to the importance of low levels of HDL-C as a risk factor for coronary heart disease, much effort has been devoted to understanding the metabolic basis of the low HDL state. Current evidence indicates that LPL plays a role in regulating HDL-C levels by hydrolyzing core TG of chylomicrons and VLDL resulting in the transfer of redundant surface compo-
TABLE VI
HDL turnover in muscle specific LPL expressors

| Genotype | n  | TG mg/dl | HDL-CE mg/dl | HDL-CE FCR pools/h | HDL-CE TR units |
|----------|----|----------|--------------|--------------------|-----------------|
| L2       | 11 | 151 ± 69 (156 ± 74) | 60 ± 12 (63 ± 10) | 0.111 ± 0.020 (0.108 ± 0.013) | 6.59 ± 1.22 (6.79 ± 1.20) |
| L2-MCK   | 13 | 74 ± 20 (79 ± 18) | 54 ± 7 (55 ± 8) | 0.121 ± 0.013 (0.120 ± 0.008) | 6.47 ± 1.17 (6.58 ± 1.19) |
| L0-MCK   | 13 | 110 ± 27 (112 ± 28) | 49 ± 4 (48 ± 4) | 0.130 ± 0.030 (0.130 ± 0.016) | 6.31 ± 1.06 (6.30 ± 0.86) |

*p values

1 L2 vs. L2-MCK | 0.001 (0.005) | 0.09 (0.04) | 0.14 (0.02) | NS* (NS) |
2 L2 vs. L0-MCK | 0.05 (0.08) | 0.002 (0.00004) | 0.02 (0.003) | NS (NS) |
3 L2-MCK vs. L0-MCK | 0.001 (0.005) | 0.05 (0.03) | 0.16 (0.08) | NS (NS) |

*NS, not significant (p > 0.4).

LPL, lipoprotein lipase; HDL, high-density lipoprotein; TG, triglyceride;
**HDL-C, high-density lipoprotein cholesterol; FCR, fractional catabolic rates; TR, transport rates.

**HDL-CE FCR were calculated using the Matthews method (57), and transport rates (TR) were estimated as FCR multiplied by HDL-CE pool size. Data in parentheses are corrected for outliers by removal of highest and lowest value in each group.
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