Inactive Lipoprotein Lipase (LPL) Alone Increases Selective Cholesterol Ester Uptake in Vivo, Whereas in the Presence of Active LPL It Also Increases Triglyceride Hydrolysis and Whole Particle Lipoprotein Uptake*

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We have previously shown that transgenic expression of catalytically inactive lipoprotein lipase (LPL) in muscle (Mck-N-LPL) enhances triglyceride hydrolysis as well as whole particle lipoprotein and selective cholesterol ester uptake. In the current study, we have examined whether these functions can be performed by inactive LPL alone or require the presence of active LPL expressed in the same tissue. To study inactive LPL in the presence of active LPL in the same tissue, the Mck-N-LPL transgene was bred onto the heterozygous LPL-deficient (LPL0/He-LPL). At 18 h of age, Mck-N-LPL reduced triglycerides by 35% and markedly increased muscle lipid droplets. In adult mice, it reduced triglycerides by 40% and increased lipoprotein particle uptake into muscle by 60% and cholesterol ester uptake by 110%. To study inactive LPL alone, the Mck-N-LPL transgene was bred onto the LPL-deficient (LPL0) background. These mice die at ~24 h of age. At 18 h of age, in the absence of active LPL, inactive LPL expression did not diminish triglycerides nor did it result in the accumulation of muscle lipid droplets. To study inactive LPL in the absence of active LPL in the same tissue in adult animals, the Mck-N-LPL transgene was bred onto mice that only expressed active LPL in the heart (LPL0/He-LPL). In this case, Mck-N-LPL did not reduce triglycerides or increase the uptake of lipoprotein particles but did increase muscle uptake of chylomicron and very low density lipoprotein cholesterol ester by 40%. Thus, in the presence of active LPL in the same tissue, inactive LPL augments triglyceride hydrolysis and increases whole particle triglyceride-rich lipoprotein and selective cholesterol ester uptake. In the absence of active LPL in the same tissue, inactive LPL only mediates selective cholesterol ester uptake.

Lipoprotein lipase (LPL, EC 3.1.1.34) is a central enzyme in lipid metabolism. As a homodimer, it is bound to endothelial heparan sulfate proteoglycans especially in the capillaries of heart muscle, skeletal muscle, and adipose tissue. By hydrolysis of plasma triglyceride (TG) from chylomicrons and VLDL, the enzyme controls fatty acid uptake into tissues and releases surface components for HDL formation (for a review, see Ref. 1).

Independent of its catalytic activity, based largely on in vitro studies, it has been proposed that LPL can also act as a structural cofactor facilitating cellular uptake of whole lipoprotein particles and selective cholesterol ester uptake. Several possible mechanisms have been proposed. It was shown that LPL can bridge between lipoproteins and lipoprotein receptors, such as the LDL receptor-related protein, and in this manner enhance whole particle uptake (for a review, see Ref. 2). LPL can also bridge between lipoproteins and heparan sulfate proteoglycans (for a review, see Ref. 3), concentrating lipoproteins in the vicinity of receptors (4) or resulting in lipoprotein internalization during the process of cell surface proteoglycan internalization (5). In addition, LPL is able to mediate selective cholesterol ester uptake from HDL (6) in a process that may be analogous to hepatic lipase-mediated selective cholesterol ester uptake by the liver (7).

We recently confirmed that catalytically inactive LPL can function in vivo in lipoprotein metabolism and uptake by tissues (8). Transgenic mouse lines were established that expressed mutant inactive human LPL (hLPL) driven by the muscle-specific Mck promoter (Mck-156N-LPL). These mice, which also have normal mouse LPL (mLPL) expression in muscle, had decreased total and VLDL-TG and in muscle had an 80% increase in uptake of VLDL protein and a 130% increase in uptake of VLDL cholesterol. This study indicated that inactive LPL could increase TG hydrolysis, whole particle lipoprotein uptake, and selective cholesterol ester uptake. However, in these experiments active LPL was also present in muscle, so we could not be certain which effects of inactive LPL were due to augmenting the function of active LPL expressed in the same tissue and which were properties of inactive LPL alone (8).

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The abbreviations used are: LPL, lipoprotein lipase; TG, triglyceride; hLPL, human LPL; mLPL, mouse LPL; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; NS, not significant.
This issue has been addressed in the current study. Two mouse models were generated that, in contrast to the previously described mice, express only inactive LPL in muscle but not active LPL. One model is the homozygous LPL knockout newborn mouse with and without transgenic expression of inactive LPL in the muscle. The second is the adult LPL-deficient mouse rescued from neonatal death by active heart LPL with and without transgenic expression of inactive LPL in muscle. The latter model has catalytically active hLPL in the heart but exclusively transgenic inactive LPL expression in muscle. In these models it was shown that inactive LPL alone can mediate selective cholesterol ester uptake, but the presence of active LPL in the same tissue was required for increased triglyceride hydrolysis and whole particle lipoprotein uptake.

**EXPERIMENTAL PROCEDURES**

**Breeding of Mice Expressing Exclusively Inactive LPL.—Mice with muscle-specific transgenic expression of catalytically inactive LPL (Mck-N-LPL, Ref. 8) were crossed with heterozygote LPL knockout mice (LPL+, Ref. 9).** Pups heterozygous for both LPL deficiency and the Mck-N-LPL transgene (LPL1/Mck-N-LPL) were crossed again with LPL1 mice. The following genotypes resulted from this cross: 1/8 wild type (LPL2), 1/8 wild type plus inactive LPL expression (LPL2/Mck-N-LPL), 1/4 LPL1, 1/4 LPL1/Mck-N-LPL, 1/8 LPL knockout (LPL0), and 1/8 LPL knockout plus inactive LPL expression (LPL0/Mck-N-LPL).

**Breeding of Mice Expressing Exclusively Inactive LPL in Muscle—Mice with heart-specific transgenic expression of active LPL (He-LPL) were generated using an 8-kb fragment of the proximal LPL promoter (10).** The transgene was bred onto the LPL0 background to produce mice expressing active LPL exclusively in the heart (LPL0/He-LPL). These mice were crossed with LPL1/Mck-N-LPL mice. The following genotypes were expected to result from this cross (12.5% each, Fig. 1): LPL1/Mck-N-LPL, heterozygous LPL deficiency with expression of heart-LPL without (LPL1/He-LPL) and with (LPL1/He-LPL/Mck-N-LPL) inactive LPL in the muscle, LPL0, LPL0/Mck-N-LPL, and mice expressing active LPL exclusively in the heart without (LPL0/He-LPL) and with inactive LPL in the muscle (LPL0/He-LPL/Mck-N-LPL). Littermate controls were used for all experiments. Mice were fed a regular diet with 4.5% of energy from fat; mice had free access to food and water.

**Genotyping of Induced Mutant Mice—Genotypes were determined by PCR from tail tip DNA.** To determine the genotype at the LPL locus, a previously reported 3-primer PCR was used (11). Another 3-primer PCR was established to detect transgenets for heart- and muscle-specific LPL expression by using specific upstream primers (5′-CGT TGA CGG TTA TCG TT-3′ for He-LPL and 5′-CAG AGG GCC TGC CCG GTC ACA TCA AG-3′ for Mck-N-LPL) with a common downstream primer (5′-GTT ACC GTC CAG CCA TGC AG-3′). This PCR resulted in a 646-bp (He-LPL) and a 477-bp (Mck-N-LPL) PCR product.

**RNA Analysis for Tissue-specific Expression of the Transgene—Total cellular RNA was extracted from frozen tissues (12) and reverse-transcribed into cDNA using a Gene Amp RNA PCR kit (PerkinElmer Life Sciences) with a random primer mix.** From total cDNA, mLPL was detected with a PCR using two specific primers (5′-CCG AGG AAT TCT GGG CCC TGT AAC-3′ and 5′-GTG ACC GTC CAG CCTA GGG AT-CACCA-3′) resulting in a 421-bp PCR product (13). PCR from total cDNA for expression of transgenic hLPL was done with the primers 5′-GTG ACC GTC CAG CCTA GGG AT-CACCA-3′ and 5′-CTC TGC AAT CAC GGG GAT ACC-3′, one of which bound before and one after intron 3 of the LPL minigene. In the inactive LPL minigene one TaqI cutting site was removed by the mutation. Therefore, digestion of the PCR product (398 bp) from unmutated wild type LPL (as in He-LPL) yielded three digestion fragments (181, 109, and 108 bp). Digestion of the PCR product from mutated, inactive LPL yielded two fragments (290 and 108 bp). Thus, expression of active and inactive LPL mRNA could be differentiated.

**Histological Analysis and Electron Microscopy—**

**Mice tissues** from 18-h-old pups expressing no transgenic LPL, enzymatically active hLPL (MCK-LPL, Ref. 14), and enzymatically inactive hLPL (MCK-N-LPL, Ref. 8) in muscle were compared on different mLPL backgrounds. After barbiturate injection and decapitation, the carcasses were frozen in TissueTek, and 4-μm-thick cryostat sections from unfixed tissue samples were stained with hematoxylin and eosin, periodic acid-Schiff, and oil red O. Small specimens from skeletal muscle were fixed in 3% cedacolylated-buffered glutaraldehyde (pH 7.3) for 4 h, postfixed with 1% OsO4 in sodium cacodylate buffer, dehydrated, and finally embedded in Arag 100. Azure-methylene blue-stained sections membranes were examined by light microscopy. For quantification of lipid droplets, an arbitrary scale from 0 (no lipid droplets) to 4 (highest amounts of lipid droplets) was used. In addition, ultrathin sections were cut with a Reichert OMU Ultracut ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips EM 400 electron microscope at 80 kV. Histological analysis of 5-6-month-old mice was done after perfusion with phosphate-buffered saline from individual organs as described above. Immunofluorescence staining was used to show LPL expression. Muscle tissue from the upper foreleg of 3-month-old mice was frozen in 5-methylbutan, which was precooled in liquid nitrogen, and then cut into 6-μm-thick sections, fixed in cooled methanol, and blocked with 5% donkey serum, 2% bovine serum albumin, and 0.1% bovine serum albumin-C in phosphate-buffered saline, 0.5% glycine. An anti-hLPL monoclonal antibody was directly labeled using the Alexa 568 protein labeling kit (Molecular Probes, Leiden, Netherlands) according to the instructions of the manufacturer. The sections were incubated with a monoclonal antibody concentration of 1:500 (1 μg/ml). Cell nuclei were stained with 4,6-diamino-2-phenylindol. Control sections were stained with hematoxylin and eosin.

**Lipid and Lipoprotein Analysis—**

**Blood—** Blood was taken after 6 h of daytime fasting by puncture of the retro-orbital plexus. Plasma TG and cholesterol were determined using commercial kits that were adapted to microtiter plates. Lipoproteins were separated by sequential ultracentrifugation using 60 μl of plasma from individual mice, and cholesterol in the fractions was determined (15). The qualitative distribution of the plasma lipoproteins was confirmed by gradient ultracentrifugation using 200 μl of pooled plasma in a continuous KBr gradient from 1.21 to 1.0 g/ml.

**Lipoprotein Turnover and Organ Uptake Study—**

Human chylomicrons from apo-C-II-deficient donors were labeled in vitro with [1,2-3H]cholesterol oleyl ether using cholesterol ester transfer protein (provided by Dr. A. Tall, Columbia University, New York) as reported previously (13). Chylomicron apoproteins were labeled in vitro with 3H-1-tyramine cellulose (14). Chylomicrons were reisolated by double ultracentrifugation. Less than 5% of 14C label was found in chylomycin lipids. For
TABLE I

|           | n  | Triglycerides | Cholesterol |
|-----------|----|---------------|-------------|
| LPL2      | 25 | 150 ± 98      | 87 ± 21     |
| LPL2/Mck-N-LPL | 21 | 78 ± 26       | 70 ± 12     |
| p         |    | <0.005        | <0.005      |
| LPL1      | 44 | 219 ± 126     | 84 ± 16     |
| LPL1/Mck-N-LPL | 38 | 143 ± 91      | 80 ± 14     |
| p         |    | <0.01         | NS          |
| LPL0      | 21 | 4392 ± 2338   | 155 ± 63    |
| LPL0/Mck-N-LPL | 19 | 4098 ± 2187   | 172 ± 79    |
| p         |    | NS            | NS          |

Inactive LPL Mediates Selective Uptake into Muscle

FIG. 2. Electron microscopy and histology of muscle tissue of 18-h-old mice. In the presence of normal mouse LPL, inactive LPL leads to a markedly increased number of muscle lipid droplets (see arrows; A, LPL2; B, LPL2/Mck-N-LPL). This is not possible in the absence of active LPL (C, LPL0; D, LPL0/Mck-N-LPL). Magnification is 3300-fold. Insets, azure-methylene blue staining of muscle, 110-fold. Arrows, lipid droplets.

RESULTS

Newborn Mice Expressing Exclusively Inactive LPL

Breeding and Survival—To obtain mice expressing only inactive hLPL and no active mLPL, male LPL1/Mck-N-LPL mice were bred with LPL1 females. Since both LPL0 and LPL0/Mck-N-LPL mice die after about 24 h of life (8), whole litters were sacrificed 18 h after delivery. From 27 litters, 168 mice with the expected genotype ratio were born (LPL2, 14.9%; LPL2/Mck-N-LPL, 12.5%; LPL1, 26.2%; LPL1/Mck-N-LPL, 22.6%; LPL0, 12.5%; and LPL0/Mck-N-LPL, 11.3%). After birth mice of all genotypes did not show any obvious differences. At 18 h of age, LPL0 and LPL0/Mck-N-LPL mice appeared pale due to high TG levels.

Plasma Lipids—TG and cholesterol from 18-h-old mice are given in Table I. On the LPL2 background, inactive LPL reduced TG by 47% (p < 0.005) and cholesterol by 20% (p < 0.005). On the LPL1 background, TG levels were reduced by 34% (p < 0.01), and cholesterol was not significantly changed. On the LPL0 background, TG dramatically increased to over 3300-fold. Insets, azure-methylene blue staining of muscle, 110-fold.

Expression of Human and Mouse LPL—Organ-specific expression of the transgenes was confirmed by reverse transcription-PCR as shown in Fig. 3. Unmutated, active hLPL was found in the hearts of both LPL0/He-LPL and LPL0/He-LPL/Mck-N-LPL but not in muscle and adipose tissue of these mice (181- and 108-bp bands). Mutated, inactive LPL was found in the muscle and in lesser amounts in the heart in LPL1/Mck-N-LPL and in LPL0/He-LPL/Mck-N-LPL (290- and 108-bp bands). A low amount of Mck-N-LPL transgene expression in the heart is expected (19). Mouse LPL was present in heart, muscle, and adipose tissue in LPL1/Mck-N-LPL mice but not in LPL0/He-LPL and LPL0/He-LPL/Mck-N-LPL mice. Therefore, adult mouse lines expressing inactive LPL with and without active LPL in muscle were established.

Plasma Lipoprotein Profile—Lipid and lipoprotein levels were determined on 10–12-week-old mice using blood drawn

mals a few finely disperse lipid droplets are seen (1 unit). In wild type animals with inactive LPL, more droplets are present. On the LPL0 background, despite very high TG, there were no muscle lipid droplets in mice without or with inactive muscle LPL. The light microscopic observations were confirmed by electron microscopy (Fig. 2). Therefore, lipid droplet accumulation in muscle does not occur if inactive LPL alone is expressed but also requires the expression of active LPL.

Adult Mice Expressing Inactive but No Active LPL in the Muscle

Breeding and Survival—Since LPL0 mice suffer neonatal death (9, 18), it was necessary to rescue them with an LPL transgene driven by a cardiac-specific promoter to obtain adult mice with either no LPL or only inactive LPL in muscle. To generate such mice, LPL1/Mck-N-LPL and LPL0/He-LPL mice were crossed (see Fig. 1 for complete breeding strategy). Taking into consideration the neonatal death of LPL0 and LPL0/Mck-N-LPL mice, the number of mice with each of the other six expected genotypes surviving to adulthood would be 16.7% per genotype. In actual fact in the 269 pups from 52 litters that survived to adulthood the genotypes were distributed as follows: LPL1, 15.3%; LPL1/Mck-N-LPL, 12.8%; LPL1/He-LPL, 17.9%; LPL1/He-LPL/Mck-N-LPL, 18.1%; LPL0/He-LPL, 17.3%; and LPL0/He-LPL/Mck-N-LPL, 19.5%.

Histological Analysis and Electron Microscopy—Muscle tissues from 18-h-old mice expressing wild type LPL (LPL2), wild type LPL plus inactive LPL (LPL2/Mck-N-LPL), no wild type LPL (LPL0), and no wild type LPL plus inactive LPL (LPL2/Mck-N-LPL) were stained with azure-methylene blue (Fig. 2, A–D insets, respectively) and with oil red O. In wild type animals a few finely disperse lipid droplets are seen (1 unit). In wild type animals with inactive LPL, more droplets are present. On the LPL0 background, despite very high TG, there were no muscle lipid droplets in mice without or with inactive muscle LPL. The light microscopic observations were confirmed by electron microscopy (Fig. 2). Therefore, lipid droplet accumulation in muscle does not occur if inactive LPL alone is expressed but also requires the expression of active LPL.

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Plasma Lipoprotein Profile—Lipid and lipoprotein levels were determined on 10–12-week-old mice using blood drawn
after a daytime fast (Table II) and 2 h after 200-μl olive oil challenge (Table III). In fasted LPL1 mice, inactive LPL reduced plasma TG by 40% in males (p < 0.001) and by 39% in females (p < 0.001). These results for total TG were reflected in VLDL-TG levels. After gavage, TG rose by about 100% in LPL1 mice. In this situation, inactive LPL reduced TG by 50% (p < 0.02) and VLDL-TG by 52% (p < 0.01). LPL-deficient mice rescued by active heart LPL (LPL1/He-LPL) had lower TG levels than LPL1 mice in the fasted state. On this background, inactive LPL did not reduce TG or VLDL-TG. After gavage, TG rose by almost 400% in LPL1 mice. As in the fasted state, inactive LPL changed neither TG nor VLDL-TG levels on this background. Mice expressing both mLPL and active He-LPL (LPL1/He-LPL) also had lower TG and VLDL-TG levels than LPL1. In the fasted state, inactive LPL did not reduce TG and VLDL-TG on this background. However, after gavage with olive oil, inactive LPL reduced TG by 38% (p < 0.01) and VLDL-TG by 41% (p < 0.01). Total cholesterol and VLDL, LDL, and HDL cholesterol were unchanged by muscle expression of inactive LPL on any background. Sequential ultracentrifugation data were confirmed by density gradient centrifugation (data not shown). Taken together these data show that inactive LPL must be expressed along with active LPL in the same tissue to exert its TG-lowering effect.

**Metabolic Studies**—To investigate the organ uptake of lipoproteins, metabolic studies with radioactively labeled human chylomicrons from apoC-II-deficient donors were performed (Fig. 4). On the LPL1 background, Mck-N-LPL increased lipoprotein protein uptake from chylomicrons into the muscle by 60% (125I-tyramine cellobiose protein: LPL1, 8.6 ± 2.3 cpm/mg; LPL1/Mck-N-LPL, 13.7 ± 2.6 cpm/mg; p < 0.005) and increased cholesterol ester uptake into muscle by 109% ([3H]cholesterol ether: LPL1, 0.37 ± 0.2 cpm/mg; LPL1/Mck-N-LPL, 0.71 ± 0.2 cpm/mg; p < 0.005). The presence of inactive LPL in muscle did not influence spleen, adipose tissue or heart 125I-tyramine cellobiose protein or [3H]cholesterol ether uptake. On the LPL1/He-LPL background without any active LPL in the muscle, Mck-N-LPL did not increase lipoprotein protein uptake.
into muscle (125I-tyramine cellobiose protein: LPL0/He-LPL, 6.6 ± 1.3 cpm/mg; LPL0/He-LPL/Mck-N-LPL, 7.3 ± 1.9 cpm/mg; p = NS). However, on the LPL0/He-LPL background, Mck-N-LPL did increase the uptake of cholesterol ester into muscle by 41% ([3H]cholesterol ether: LPL0/He-LPL, 0.63 ± 0.09 cpm/mg; LPL0/He-LPL/Mck-N-LPL, 0.96 ± 0.23 cpm/mg; p < 0.005). Again the presence of inactive LPL in muscle did not influence spleen, adipose tissue, or heart uptake. On the LPL1/He-LPL background, Mck-N-LPL mediated a 66% increased uptake of 125I-tyramine cellobiose protein (p < 0.05) and a 95% increased uptake of [3H]cholesterol ether (p < 0.005) into the muscle. On this background, the presence of inactive LPL in muscle also did not influence spleen, adipose tissue, or heart uptake. The results with doubly labeled chylomicrons were confirmed with doubly labeled VLDL. On the LPL0/He-LPL background, Mck-N-LPL did not increase muscle lipoprotein protein uptake from VLDL (125I-tyramine cellobiose protein: LPL0/He-LPL, 1.13 ± 0.4 cpm/mg; LPL0/He-LPL/Mck-N-LPL, 1.25 ± 0.3 cpm/mg; p = NS), whereas cholesterol ester uptake was increased by 64% ([3H]cholesterol ether: LPL0/He-LPL, 0.36 ± 0.1; LPL0/He-LPL/Mck-N-LPL, 0.58 ± 0.17 cpm/mg; p < 0.02). Therefore, inactive LPL in muscle along with active LPL augments lipoprotein particle and selective cholesterol ester uptake. However, in the absence of active LPL in the same organ, inactive LPL increases only selective cholesterol ester uptake but not whole particle uptake.

**Muscle Histology**—As shown in Fig. 5, 6-month-old LPL1 mice and LPL0/He-LPL mice have normal muscle histology after routine and periodic acid-Schiff staining (Fig. 5, A and C). However, the addition of the Mck-N-LPL transgene on both of

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**TABLE III**

**Plasma lipoprotein levels in adult mice after oral lipid load**

|                  | TG     | VLDL-TG | TC     | VLDL-C | LDL-C | HDL-C |
|------------------|--------|---------|--------|--------|-------|-------|
| LPL1             | 218 ± 115 | 160 ± 79 | 91 ± 12 | 23 ± 24.8 | 18 ± 4.1 | 50 ± 7.8 |
| LPL0/He-LPL      | 110 ± 54  | 77 ± 39  | 99 ± 6.4 | 22 ± 3.1  | 22 ± 5.9 | 54 ± 6.7 |
| p                | <0.02   | <0.01   | NS     | NS      | NS     | NS     |
| LPL0/He/LPL/Mck-N-LPL | 254 ± 120 | 194 ± 95 | 111 ± 10 | 27 ± 5.4  | 25 ± 3.5 | 61 ± 7.3 |
| p                | NS      | NS      | NS     | NS      | NS     | NS     |
| LPL0/He-LPL      | 143 ± 45  | 96 ± 35  | 94 ± 19  | 19 ± 3.5  | 17 ± 4.0 | 50 ± 11 |
| LPL0/He/LPL/Mck-N-LPL | 89 ± 21   | 57 ± 14  | 110 ± 43 | 18 ± 4.5  | 16 ± 4.2 | 52 ± 10 |
| p                | <0.01   | <0.01   | NS     | NS      | NS     | NS     |

**FIG. 4.** Chylomicron organ uptake and selective cholesterol ester uptake. In the absence of active LPL in the muscle, inactive LPL augments selective cholesterol ester uptake but not particle uptake. Human chylomicrons from apoC-II-deficient donors were labeled at apolipoproteins with 125I-tyramine cellobiose and in the cholesterol ester core with [3H]cholesterol ether. 1 h after injection of the label, mice were perfused, and organs were isolated and counted for 125I. [3H] radioactivity was determined from a lipid extract of the total sample. Data are given as mean ± S.D. Both 125I and [3H] radioactivities were set to 100% in LPL1 and in LPL0/He-LPL mice, respectively. Shown are protein uptake (A) and cholesterol ester uptake (B) on the LPL1 background and protein uptake (C) and cholesterol ester uptake (D) on the LPL0/He-LPL background. AT, adipose tissue. ***, p < 0.005.
these backgrounds resulted in increased numbers of muscle fibers with centralized nuclei, pathological periodic acid-Schiff-positive staining (glycogen), and nonspecific signs of muscle damage (Fig. 5, B and D). In data not shown, muscle with inactive LPL also had increased numbers of mitochondria-rich muscle fibers as shown by azure-methylene blue-stained semithin sections, but there was no increase in neutral lipid storage by oil red O staining. Therefore, expression of inactive LPL without active LPL in muscle can cause myopathic histological changes, suggesting these may be due to selective cholesterol ester uptake rather than whole particle lipoprotein uptake by muscle.

**Immunfluorescence Analysis**—The cellular distribution in muscle of inactive LPL expressed by the Mck-N-LPL transgene was studied by immunofluorescence, and the results are shown in Fig. 6. The presence of the inactive LPL was associated with staining of the plasma membrane of muscle cells and the blood vessel wall. This indicates that the Mck-N-LPL transgene causes the expression of LPL capable of reaching the correct locations for it to exert its metabolic effects.

**DISCUSSION**

The purpose of this study was to further explore the nonenzymatic functions of LPL in lipid and lipoprotein metabolism in an *in vivo* system. Previously we reported a transgenic mouse line expressing catalytically inactive LPL in muscle (8). The mutant LPL in the transgene had an Asp156 to Asn substitution, which had originally been found in LPL-deficient patients (20). It was capable of normal binding to both heparin and lipoproteins *in vitro* (21) and was found to assume a normal dimeric conformation and have normal proteoglycan binding *in vivo* (8). The expression of this mutant LPL in muscle resulted in decreased TG levels and increased lipoprotein whole particle and selective cholesterol ester uptake (8). A major problem in the interpretation of the results of these experiments was the simultaneous presence of active endogenous mLPL in muscle of these mice. Thus the properties of inactive LPL alone versus those of inactive LPL in concert with active LPL could not be distinguished. In the present study, two different mouse models were created that expressed only inactive LPL in muscle. It was shown that inactive muscle LPL can reduce TG and mediate lipoprotein whole particle uptake only in the presence of active LPL in the same tissue. However, inactive muscle LPL by itself can only mediate selective cholesterol ester uptake. The latter was sufficient to cause a myopathy.

One of the major results of the current study is that we observed whole lipoprotein particle uptake mediated by inactive LPL only in the presence of active LPL expression in the same tissue. In contrast, several *in vitro* studies have shown in various cell types that LPL can mediate lipoprotein uptake independent of its catalytic activity (2, 3, 22, 23). A possible explanation is that *in vivo* some active LPL is required on the capillary endothelium of muscle to decrease lipoproteins to an optimal size for making their way across the endothelial barrier (24). Alternatively lipolysis may increase the permeability of the endothelial barrier and allow passage of larger lipoproteins (25) that then bind to and are internalized by inactive LPL on the muscle plasma membrane.

When inactive LPL was expressed in the muscle together with active LPL, it lowered fasted and postprandial plasma TG levels. In newborn pups this was the case even though, in the first weeks of life, LPL expression has not reached adult levels in most peripheral tissues (26–28). Without any active LPL on the LPL0 background inactive muscle LPL did not decrease plasma TG. One possible mechanism for the TG-lowering effect is inactive LPL mediated whole lipoprotein particle uptake as discussed above. Another possible mechanism could involve hydrolysis. Since inactive LPL cannot carry out hydrolysis, it must in some way augment the function of the natural lipolytic system. Inactive LPL is capable of tethering lipoproteins to endothelial proteoglycans (3) placing them in proximity to active LPL, which does the hydrolysis. The rate-limiting step in this pathway may be the amount of LPL (active and inactive) available for tethering rather than the amount of active enzyme. Another possibility is that functional dimers form between active and inactive LPL. Such dimers with half the specific activity of homodimers of active LPL (29) may allow the available active LPL to spread over a wider surface allowing more efficient hydrolysis of large triglyceride-rich lipoproteins. Alternatively some active LPL molecules that otherwise would participate only in the binding process might be “free” to perform lipid hydrolysis. Several investigators including Olivec-rona et al. (3) and Rinninger et al. (30) have postulated that plasma lipolysis is limited by factors other than the amount of postheparin LPL activity itself. It is hypothesized that the anchoring of lipoproteins to endothelial proteoglycans, a process that can be mediated by inactive LPL, is the rate-limiting process in LPL-mediated hydrolysis; the amount of active LPL, provided it is present at all, might be less important.

Another finding of this study is that inactive LPL in muscle can mediate selective cholesterol ester uptake independent of the presence of active LPL. It is possible that triglyceride-rich lipoproteins or their remnants trapped at the capillary endo-

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**Fig. 5. Muscle histology of adult mice.** Overexpression of inactive LPL in muscle leads to a myopathy (pathological glycogen accumulation and increased number of centralized nuclei; arrows in B and D) independent of the presence of active LPL. Periodic acid-Schiff staining, magnification ×113, of femoral muscles from 6-month-old mice with different genotypes: A, LPL1; B, LPL1/Mck-N-LPL; C, LPL0/He-LPL; D, LPL0/He-LPL/Mck-N-LPL.

**Fig. 6. LPL immunofluorescence in skeletal muscle.** Immunofluorescence using a monoclonal antibody against hLPL shows localization of transgenic LPL (red staining) in the plasma membrane of muscle cells and in the blood vessel wall. A, LPL0/He-LPL; B, LPL0/He-LPL/Mck-N-LPL. Big arrow, blood vessel; small arrow, endomysial capillary. Bars denote 50 μm.
Inactive LPL Mediates Selective Uptake into Muscle

In summary, we show in two mouse models that inactive LPL alone mediates selective cholesterol ester uptake into muscle tissue from chylomicrons and VLDL and that this can result in myopathic changes. In addition, we show that inactive LPL in the presence of active LPL in the same tissue can also decrease triglycerides and increase whole particle lipoprotein uptake into muscle.

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Inactive Lipoprotein Lipase (LPL) Alone Increases Selective Cholesterol Ester Uptake in Vivo, Whereas in the Presence of Active LPL It Also Increases Triglyceride Hydrolysis and Whole Particle Lipoprotein Uptake

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