Lipoprotein lipase-facilitated uptake of LDL is mediated by the LDL receptor

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Abstract LPL mediates the uptake of lipoproteins into different cell types independent of its catalytic activity. The mechanism of this process and its physiological relevance are not clear. Taking into account the importance of the endothelial barrier for lipoprotein uptake, in vitro studies with primary aortic endothelial cells from wild-type and low density lipoprotein receptor (LDLR)-deficient ($LDLR^{-/-}$) mice were performed. Addition of LPL almost doubled the uptake of LDL into wild-type cells. However, there was virtually no LPL-mediated change of LDL uptake into $LDLR^{-/-}$ cells. Upregulation of LDLR by lipoprotein-deficient serum/lovastatin in wild-type cells resulted in a 7-fold increase of LPL-mediated LDL uptake. Uptake of chylomicron remnants was not affected by LDLR expression. In proteoglycan-deficient cells, LPL did not increase the uptake of lipoproteins. The physiological relevance of this pathway was studied in mice that were both $LDLR^{-/-}$ and transgenic for catalytically inactive LPL in muscle. In the presence of LDLR, inactive LPL reduced LDL cholesterol significantly (13–24%). In the absence of LDLR, LDL cholesterol was not affected by transgenic LPL. Metabolic studies showed that in the presence of LDLR, LPL increased the muscular uptake of LDL by 77%. In the absence of LDLR, transgenic LPL did not augment LDL uptake. Chylomicron uptake was not affected by the LDLR genotype. We conclude that LPL-mediated cellular uptake of LDL, but not of chylomicrons, is dependent on the presence of both LDLR and proteoglycans.— Loeffler, B., J. Heeren, M. Blaeser, H. Radner, D. Kayser, B. Aydin, and M. Merkel. **Lipoprotein lipase-facilitated uptake of LDL is mediated by the LDL receptor. *J. Lipid Res.* 2007.** 48: **288–298.**

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Lipoprotein lipase (EC 3.1.1.34) is a central enzyme in plasma lipid metabolism. Bound to endothelial heparan sulfate proteoglycans (HSPGs) primarily in the capillaries of cardiac muscle, skeletal muscle, and adipose tissue, it hydrolyzes triglycerides (TGs) from chylomicrons and VLDLs, controls fatty acid uptake into tissues, and releases components for HDL formation. Mutations in the LPL gene have been linked to human diseases such as hypertriglyceridemia, familial combined hyperlipoproteinemia, metabolic syndrome, and cardiovascular disease (reviewed in Ref. 1).

Independent of its catalytic activity, LPL acts as a structural factor facilitating the cellular uptake of lipoprotein particles and cholesteryl ester. Based on in vitro studies, several mechanisms have been proposed for this process. It was found that LPL can bridge between lipoproteins and receptors with consecutively increased cellular uptake of whole lipoproteins (reviewed in Ref. 2). In addition, LPL enhances binding between lipoproteins and HSPGs (reviewed in Ref. 3), resulting in a concentration of lipoproteins in the vicinity of receptors (4) or in lipoprotein internalization during physiological HSPG recycling (5).

Recent studies have shown that LPL-mediated lipoprotein uptake also occurs in vivo. Transgenic mice expressing mutant inactive LPL in skeletal muscle had decreased plasma TG, faster turnover of TG-rich lipoproteins, and increased muscle uptake of whole lipoproteins, lipoprotein-derived fatty acids, and cholesteryl ester (6). Inactive LPL augmentation of TG hydrolysis and lipoprotein uptake, but not of selective cholesteryl ester uptake, was dependent on the simultaneous presence of normal active LPL in the same organ (7). Furthermore, LPL was found to be a structural component of remnant particles, facilitating their hepatic clearance (8).

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Abbreviations: ATCC, American Type Culture Collection; FCS, fetal calf serum; HSPG, heparan sulfate proteoglycan; LDLR, low density lipoprotein receptor; LPDS, lipoprotein-deficient serum; LRP1, LDLR-related protein 1; Mck-N-LPL, transgenic expression of inactive lipoprotein lipase in muscle; TG, triglyceride; THL, tetrahydrolipostatin; VLDLR, very low density lipoprotein receptor.

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The function of lipoprotein receptors in LPL-mediated lipoprotein uptake has been investigated in several in vitro studies. It was shown that LPL can bind and enhance lipoprotein uptake via receptors of the low density lipoprotein receptor (LDLR) family (2). Such LPL-receptor interactions with very low density lipoprotein receptor (VLDLR) (9, 10), gp330 (11), and LDLR-related protein 1 (LRP1) (12, 13) are undisputed. However, questions remain concerning the function of the LDLR during LPL-meditated lipoprotein uptake. Although LPL has been reported by some investigators to not bind to LDLR (5, 12), LPL might still increase the cellular uptake of lipoproteins via the LDLR (14). A recent study suggested that lipid rafts are involved in this process (15).

In previous studies, mostly human hepatocytes, fibroblasts, or established macrophage and endothelial cell lines were used. However, the development of inbred genetically modified mouse lines allows the use of altered primary cells. Therefore, in this study, we used primary aortic endothelial cells from inbred mouse lines with different LDLR genotypes to investigate the role of LDLR in the endothelial barrier for LPL-mediated lipoprotein uptake. The physiological significance of this pathway was confirmed by studies in an LDLR-deficient ($LDLR^{-/-}$) mouse line with transgenic expression of catalytically inactive LPL in muscle. It was found that LPL-mediated uptake of LDL, but not of chylomicrons, was dependent on the presence of both proteoglycans and the LDLR.

METHODS

Mice

C57BL/6J and $LDLR^{-/-}$ (16) mice were from Jackson Laboratory (Bar Harbor, ME). $LDLR^{-/-}$ mice had been backcrossed for 15 generations to the C57BL/6 background. Mice with muscle-specific transgenic expression of catalytically inactive LPL (Mck-N-LPL) (6) were crossed to the $LDLR^{-/-}$ background. The final cross resulted in all expected genotypes (see Results). Mice were kept on a 12 h light cycle with free access to diet and water in a specific pathogen-free animal facility. For some studies, 4–5 month old mice were fed a Western-type diet for 4 weeks (17). Animal experiments were performed in accordance with Federation of American Societies for Experimental Biology guidelines and approved by the Department of Veterinary Affairs of the State of Hamburg. Genotypes were determined from tail tip DNA using previously reported PCR techniques for the respective genotypes (7, 16).

Cell lines

Chinese hamster ovary cells (CHOK1 and CHO745) were cultivated in Ham's-F12 nutrient mixture with L-glutamine supplemented with 10% fetal calf serum (FCS) and 100 U/ml penicillin/streptomycin. Mouse embryonic fibroblasts (MEF1) were cultured in DMEM with GlutaMAX I, 4,500 mg/l D-glucose, sodium pyruvate, 10% FCS, and 100 U/ml penicillin/streptomycin. Murine endothelial cells [MS1; American Type Culture Collection (ATCC) No. CRL-2279] and human epithelial cells (HeLa; ATCC No. CCL-2) were cultivated according to ATCC recommendations. Trypsin-EDTA solution contained 0.5 g/l trypsin and 0.2 g/l EDTA. Cells were cultured in a CO_2 incubator at 37°C.

Isolation and cultivation of primary endothelial cells

For each experiment, five male mice per genotype were euthanized. After flushing the aorta with 1 ml of 37°C HBSS through the cardiac apex, thoracic aorta was dissected under aseptic conditions and placed into warm endothelial cell medium (DMEM with 20% FCS, 2 mmol/l L-glutamine, 2 mmol/l sodium pyruvate, 20 mmol/l HEPES, 1% nonessential amino acids, 100 U/ml penicillin/streptomycin, 90 μg/ml heparin, and 100 μg/ml endothelial cell growth supplement; Bioscience) (18). Connective tissue was carefully removed. Six-well plates were prepared by incubation with BD Matrigel Matrix (Bioscience) at 37°C for 30 min (19). Aortas were cut into 2–3 mm rings and placed into Matrigel with 2 ml of endothelial cell medium. After 5–7 days, explants were removed. On day 8, cells were dispensed from the Matrigel using Dispase (Bioscience) incubation for 3 min. Cells were gently resuspended in Dispase/Matrigel mixture, and 9 cm cell culture wells were precoated with this mix. Cells were used for experiments at 50–80% confluence.

RNA isolation and real-time RT-PCR

Total RNA was isolated using the NucleoSpin RNAII kit (Macherey-Nagel, Düren, Germany). Extracted RNA was dissolved in diethylpyrocarbonate-treated water and subjected to reverse transcription using a first-strand cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden). RT-PCR was performed with a Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) using a commercially available master mix containing Taq DNA polymerase and SYBR-Green I deoxynucleoside triphosphates (Roche Molecular Biochemicals). PCR conditions were as follows: primers were 5′-ATCCATATGCATCCCCAGTCTTTGG-3′ and 5′-GGATACACTCACTGCTACCACAGTG-3′ for LDLR, 5′-CCTGCACCGAGCGCA-3′ and 5′-GTCCCTGTGGACCTGCATTC-3′ for CD34, and 5′-CCAGGATAAGGAAGGAATTCCTG-3′ and 5′-CCAGCACCACATTCATCAGAAGG-3′ for S27 at 0.25 pmol/l, with 4 mmol/l $MgCl_2$, and 45 cycles of 1 s at 94°C, 10 s at 58°C, and 10 s at 72°C.

Fluorescence-activated cell-sorting analysis of flk1 (vascular endothelial growth factor receptor 2) expression

Cells were washed with PBS, detached with 0.5 mM EDTA/PBS, and washed again with PBS/3% FCS. A total of 10^5 cells were incubated for 1 h at 4°C with anti-flk1 polyclonal antibody (rabbit; Santa Cruz, CA; 1:25 in PBS/3% FCS) and then for 45 min at 4°C with Cy2-labeled anti-rabbit IgG (goat; Dianova, Hamburg, Germany; 1:200 in PBS/3% FCS). Then, cells were washed, resuspended, and analyzed (20).

Fluorescence uptake experiments

Cells were seeded on 0.1% gelatin-coated glass coverslips and incubated for 2 h at 37°C with 1 μg/ml Alexa Fluor 488AcLDL (Molecular Probes, Karlsruhe, Germany) in DMEM. Cells were washed and fixed for 30 min with PBS/4% paraformaldehyde at room temperature. After washing and nuclear staining with 4′,6-diamino-phenylindole (Sigma-Aldrich, Steinheim, Germany), coverslips were photographed using a Zeiss Axiovert 100 microscope.

Colocalization experiments

Cells grown on 0.1% gelatin-coated glass coverslips incubated with Cy5 (Amersham)-labeled LDL (20 μg/ml) and suspended in DMEM containing 1% BSA at 37°C for 20 min. Surface-bound LDL was released by 500 U/ml heparin at 4°C for 15 min. Then, cells were fixed for 2 min at room temperature in PBS/4% paraformaldehyde and permeabilized with methanol at −20°C

for 5 min. After washing with PBS, blocking was performed with 1% BSA, 10% goat serum, and 0.02 mol/l glycine in PBS. LDLR was stained by incubation for 20 min at 37°C with an Alexa Fluor 488 (Molecular Probes)-labeled chicken polyclonal antibody (Progen, Heidelberg, Germany) diluted in DMEM/1% BSA. After washing with PBS and nuclear staining with 4′,6-diaminophenylindole, mounted coverslips were photographed.

Radioactive uptake experiments

A total of 5×10^4 cells per well were plated on 0.1% gelatin-coated 12-well dishes and grown to 50% confluence. After washing with PBS, cells were incubated with 500 μl of radioactively labeled lipoproteins (5 μg/ml ^{125}I-chylomicron remnants or 20 μg/ml ^{125}I-LDL in DMEM/2% FCS) with and without 1 μg/ml LPL inactivated with tetrahydrolipostatin (THL; 1:2,000). After incubation for 1 h at 37°C, medium was removed and replaced with ice-cold PBS. After washing, cells were incubated for 10 min with 500 μl of chilled PBS/100 U/ml heparin to release surface-bound lipoproteins. Medium was removed and counted. Cells were destroyed with 500 μl of 0.1 N NaOH for 30 min, and radioactivity was counted. Protein concentration was measured using the Bio-Rad protein assay (Bio-Rad Laboratories GmbH; modified as in Ref. 21). Human chylomicron remnants were produced from human apolipoprotein C-II-deficient donor chylomicrons as reported (8). Isolation of human LDL and radioactive labeling of lipoproteins were performed according to Goldstein, Basu, and Brown (22).

LDLR upregulation

Cells were treated with medium supplemented with 10% lipoprotein-deficient serum (LPDS) instead of FCS (22). After 24 h, 1 μg/ml lovastatin (Sigma-Aldrich) was added for 24 h (14).

Western blotting

After washing, cells were lysated for 20 min on ice in 50 mM Tris, pH 8.0, 2 mM $CaCl_2$, 80 mM NaCl, 1% Triton X-100, and 1:1,000 protease inhibitor complex (Calbiochem). Lysates were cleared by centrifugation, and protein concentration was measured. The supernatants were subjected to 10% SDS-PAGE, immunoblotted for LRP1 or VLDLR, and detected using the ECL system (Amersham Biosciences). Primary antibodies were anti-LRP1 monoclonal (mouse; Progen) and anti-VLDLR polyclonal (23). Secondary antibody was HRP-labeled anti-IgG (sheep; Amersham, Pharmacia).

Lipid and lipoprotein analysis in mice

Blood was taken after 6 h of daytime fasting by retro-orbital puncture. Plasma TG and cholesterol were determined using commercial kits (Boehringer Mannheim) that were adapted to microtiter plates. Lipoproteins were separated by sequential ultracentrifugation using 60 μl of plasma from individual mice (24). Qualitative distribution of plasma lipoproteins was confirmed by gradient ultracentrifugation using 200 μl of pooled plasma per genotype in a continuous 2 ml KBr gradient from 1.21 to 1.0 g/ml. Data from three to four gradients per genotype were pooled.

Lipoprotein turnover and organ uptake study

Human chylomicrons or LDL apolipoproteins were labeled in vitro with ^{125}I-tyramine cellobiose as described (25). Lipoproteins were reisolated by double ultracentrifugation and dialyzed against PBS. Less than 1% of ^{125}I label was found in lipid extracts (26). For turnover studies, anesthetized mice (seven to eight per group) were injected into their tail vein with 2×10^6 cpm ^{125}I-tyramine cellobiose lipoproteins. Plasma turnover of lipoproteins was determined from 15 μl of plasma (chylomicrons: 2, 5, 10, 20, 30, 45, and 60 min; LDL, 2, 5, 12.5, 30, 90, 300, and 1,200 min after injection). After anesthesia, carcasses were perfused with 10 ml of PBS containing 10 units of heparin. Radioactivity of plasma and weighed organs was counted.

Muscle lipid determination

Perfused muscles of 5–6 month old mice were homogenized in 20 mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$, and 0.25 M sucrose. Homogenates were centrifuged twice for 15 min at 800 *g*, and cholesterol, TG, and phospholipids were determined by standard colorimetric assays (Roche and Wako). Protein concentrations were measured according to Ref. 27, as modified for lipid-containing samples by the addition of 0.1% SDS.

Histological analysis

Five to 6 month old mice were perfused with PBS, and femoral muscles were frozen in TissueTek. Cryocut sections (4 μm) from unfixed tissue samples were stained with hematoxylin-eosin, periodic acid Schiff, and Oil Red O.

Statistical analysis

Unless stated otherwise, results are means ± SD. Statistical significance was tested using a two-tailed Student's *t*-test. Analysis of turnover studies was made using Prism (GraphPad Software, San Diego, CA).

RESULTS

The function of LDLR during LPL-mediated lipoprotein uptake has been addressed by a combination of in vitro and in vivo experiments. Lipoprotein uptake studies were performed using primary aortic endothelial cells from inbred mice with different LDLR genotypes. The physiological significance of the results was tested by studying an LDLR$^{-/-}$ mouse line transgenically expressing catalytically inactive LPL in myocytes.

Mouse breeding

Mice with muscle-specific expression of inactive LPL (6) were crossed onto the LDLR$^{-/-}$ background. In the final cross, all expected genotypes were found: heterozygous LDLR-deficient mice without (LDLR$^{+/-}$) and with (LDLR$^{+/-}$Mck-N-LPL) inactive LPL expression, and homozygous LDLR$^{-/-}$ mice without (LDLR$^{-/-}$) and with (LDLR$^{-/-}$Mck-N-LPL) inactive LPL expression. No difference in vitality or survival was observed up to 6 months of age.

Characterization of murine primary aortic endothelial cells

Aortic primary endothelial cells were characterized for several endothelial markers and functions. Compared with control cells (MEF1), primary cells from wild-type and LDLR$^{-/-}$ mice had a several-fold higher expression of CD34, a specific marker for endothelial cells (**Fig. 1A**, left panel) (quantitative RT-PCR: MEF1, 1.0 arbitrary unit;

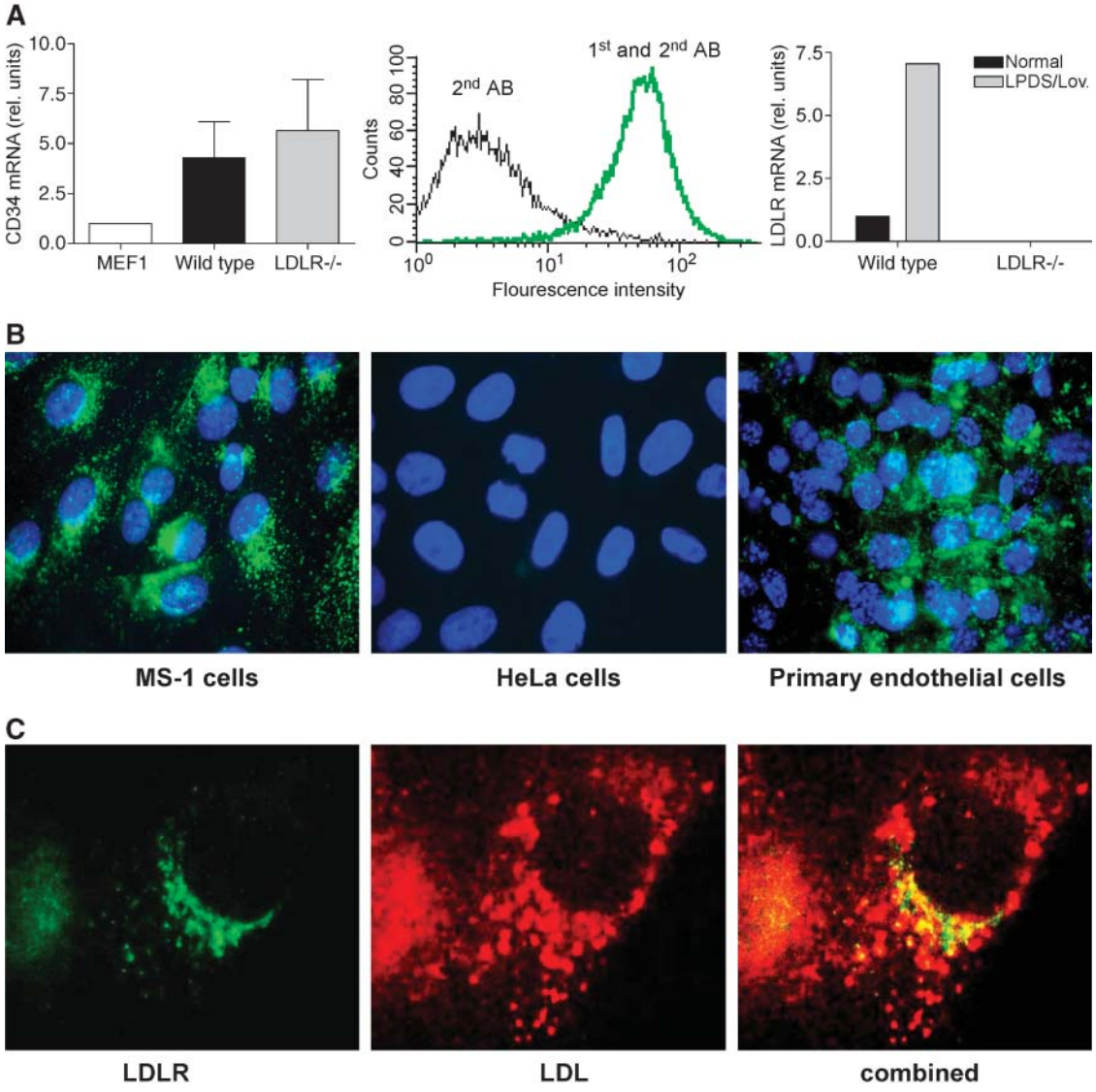

Fig. 1. Characterization of isolated primary endothelial cells. Primary cells were characterized using several markers. A: Gene expression of primary endothelial cells. Left panel: Expression of CD34 by quantitative PCR in wild-type and low density lipoprotein receptor (LDLR)-deficient ($LDLR^{-/-}$) cells. The control was the mouse embryonic fibroblast (MEF1) line. Values shown are means ± SD. Middle panel: Expression of vascular endothelial growth factor receptor 2 (VEGFR2/flk1) by fluorescence-activated cell-sorting analysis. AB, antibody. Right panel: Expression of LDLR by quantitative PCR in wild-type and $LDLR^{-/-}$ cells in normal and lipoprotein-deficient serum (LPDS)/lovastatin (Lov.) medium. B: Identification of scavenger receptor in primary endothelium by uptake of fluorescence-labeled Alexa Fluor 488AcLDL. The positive control was MS1; the negative control was the human epithelial cell line (HeLa). C: Analysis of colocalization of LDLR and LDL by immunofluorescence in wild-type cells (yellow stain in right panel).

wild-type cells, 4.3 units; $LDLR^{-/-}$ cells, 5.6 units). The absence of nonendothelial cells in culture was assessed by analysis of the expression of the vascular endothelial growth factor receptor 2 (VEGFR2/flk1). Fluorescence-activated cell-sorting analysis revealed that >90% of the isolated cells were positive for VEGFR2/flk1 (Fig. 1A, center panel), showing that <10% of the cultured cells were not of endothelial origin. LDLR expression was examined by quantitative RT-PCR in wild-type and $LDLR^{-/-}$ cells cultured in normal medium and in medium supplemented with LPDS and lovastatin. As anticipated, LDLR was detected only in wild-type cells. LPDS/lovastatin treatment resulted in a 7-fold upregulation of LDLR (Fig. 1A, right panel). The presence of scavenger receptor was proven by the uptake of fluorescence-labeled Alexa Fluor 488AcLDL (green label in Fig. 1B; positive control, mouse endothelial cell line MS1; negative control, human HeLa cells). Uptake of LDL particles into primary endothelial cells specifically by LDLR was shown by colocalization of fluorescence-labeled LDL particles (green) and LDLR (red) (Fig. 1C, right panel). Thus, it was shown by several methods that primary cells isolated from mouse aorta had endothelial characteristics. In addition, there was no significant contamination with other cell types.

Uptake of lipoproteins into primary endothelial cells

In wild-type cells, THL-inactivated LPL was able to increase the uptake of both chylomicron remnants (no LPL, 100 ± 33%; with LPL, 220 ± 25%) (**Fig. 2A**) and LDL (no LPL, 100 ± 12%; with LPL, 190 ± 65%) (Fig. 2B). Upregulation of LDLR by LPDS/lovastatin did not change chylomicron remnant uptake into wild-type cells (no LPL, 126 ± 22%; with LPL, 188 ± 34%) (Fig. 2A). However, upregulation of LDLR resulted in 3-fold increased LDL uptake compared with nonupregulated cells (no LPL, 290 ± 30%) (Fig. 2B). Addition of LPL in this situation increased LDL uptake by 7-fold (684 ± 139%) (Fig. 2B). In $LDLR^{-/-}$ cells, chylomicrons were taken up as in wild-type cells (no LPL, 123 ± 17%; with LPL, 224 ± 34%) (Fig. 2A). However, baseline uptake of LDL was markedly reduced in $LDLR^{-/-}$ cells (no LPL, 64 ± 15% compared with wild-type cells). Addition of LPL did not significantly increase LDL uptake into these cells (91 ± 55%) (Fig. 2B). Western blot analysis of VLDLR did not show differences between the genotypes, whereas analysis of LDL receptor-related protein (LRP1) expression showed an ~2-fold overexpression in $LDLR^{-/-}$ cells compared with wild-type cells (data not shown). It is concluded that LPL-mediated uptake of LDL, but not of chylomicron remnants, into endothelial cells is mediated by the LDLR.

LDL uptake into CHO cells with and without HSPG

CHOK1 cells with normal cell surface HSPG had >4-fold increased LDL uptake after the addition of THL-inactivated LPL (no LPL, 100 ± 18%; with LPL, 440 ± 36%) (**Fig. 3A**). After upregulation of the LDLR by LPDS/lovastatin, LPL-mediated uptake of LDL was increased even more (no LPL, 184 ± 24%; with LPL, 791 ± 35%) (Fig. 3B). However, in HSPG-deficient CHO745 cells, no significant effects of LPL on LDL uptake were observed. Therefore, it was shown again that the LDLR plays a crucial role in the LPL-mediated uptake of LDL. However, the additional presence of HSPG seems to be obligatory for this pathway.

Plasma lipoprotein profile in mice

Plasma lipoproteins were analyzed by sequential and gradient ultracentrifugation. On a chow diet (**Table 1**) in the presence of LDLR ($LDLR^{+/-}$ background), inactive LPL reduced LDL cholesterol by 24% (males; $P < 0.0005$) and 13% (females; $P < 0.01$). In males, this resulted in a significant reduction of total cholesterol by 16% ($P < 0.005$). LDL-TG was reduced by inactive LPL in males but not in females. As expected, plasma non-HDL lipoproteins were increased in the absence of the LDLR ($LDLR^{-/-}$ background). On this background, LDL cholesterol was not influenced by transgenic inactive LPL in male and female mice. However, in the presence of inactive LPL in muscle, VLDL-TG was significantly increased in both males (33%; $P < 0.005$) and females (29%; $P < 0.01$) on this background. Gradient centrifugation with pooled plasma confirmed the changes in the lipoprotein profile (**Fig. 4**). $LDLR^{+/-}$Mck-N-LPL mice had a markedly reduced LDL peak compared with $LDLR^{+/-}$ mice. No difference was seen in the cholesterol gradient profiles between $LDLR^{-/-}$ and $LDLR^{-/-}$Mck-N-LPL mice. Lipoprotein values after 4 weeks on a Western type diet are shown in **Table 2**. As expected (16, 28), both VLDL and LDL cholesterol levels were significantly higher than those of chow-fed mice. Again, transgenic inactive LPL reduced LDL cholesterol on the heterozygote, but not on the homozygote, LDLR-deficient background. However, these changes were not as dramatic as those found on the chow diet. On the $LDLR^{-/-}$ background, transgenic expression of inactive LPL also resulted in increased VLDL-TG levels.

Metabolic studies

Turnover studies were performed with radioactively labeled chylomicrons and LDL. The half-life of ^{125}I-TC chylomicrons was not affected by the presence or absence of the LDLR or transgenic mutated LPL (data not shown). Uptake of chylomicrons into muscle was increased significantly by inactive LPL both on the $LDLR^{+/-}$ (by 63%) and on the $LDLR^{-/-}$ (by 52%) background [$LDLR^{+/-}$,

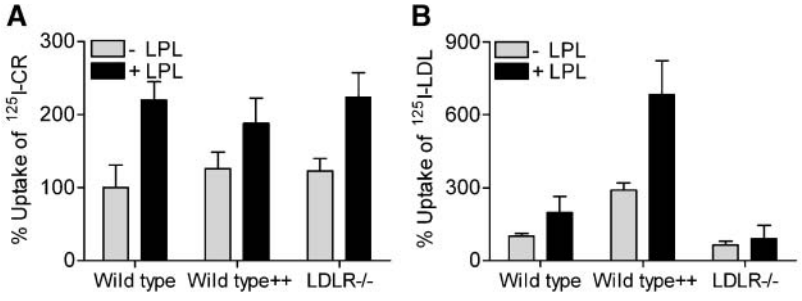

Fig. 2. Uptake of lipoproteins into primary endothelial cells. ^{125}I-labeled human chylomicron remnants (CR; 5 μg/ml; A) or ^{125}I-labeled LDL (20 μg/ml; B) was added in the absence and presence of 1 μg/ml tetrahydrolipostatin (THL)-inactivated LPL. Uptake into wild-type cells was investigated with both normal and LPDS/lovastatin (++) medium. After removing surface-bound lipoproteins by heparin treatment, cells were lysated and lipoprotein uptake per milligram of cell protein was estimated. LPL-mediated uptake of LDL, but not of chylomicron remnants, was dependent on the presence and expression level of the LDLR. Values shown are means ± SD.

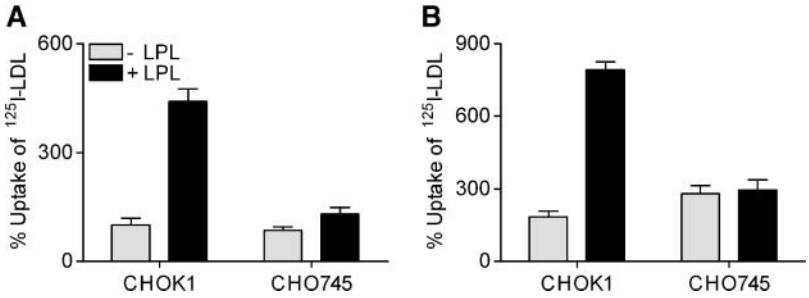

Fig. 3. Uptake of LDL in normal (CHOK1) and heparan sulfate proteoglycan (HSPG)-deficient (CHO745) cells. ^{125}I-labeled human LDL (20 μg/ml) was added to cultured cells in the absence and presence of 1 μg/ml THL-inactivated LPL. After removing surface-bound lipoproteins by heparin treatment, cells were lysated and lipoprotein uptake per milligram of cell protein was calculated. A: Cells grown in normal medium. B: Cells grown in medium supplemented with LPDS and lovastatin. LPL-mediated uptake of LDL was possible only in the presence of HSPG. Values shown are means ± SD.

1.9 ± 0.3 cpm/mg; LDLR$^{+/-}$Mck-N-LPL, 3.1 ± 0.3 cpm/mg ($P < 0.001$); LDLR$^{-/-}$, 2.3 ± 0.4 cpm/mg; LDLR$^{-/-}$Mck-N-LPL, 3.5 ± 0.7 cpm/mg ($P < 0.01$)]. As expected from previous studies, most radioactivity was taken up by the liver without significant differences between the genotypes (LDLR$^{+/-}$, 373 ± 43 cpm/mg; LDLR$^{+/-}$Mck-N-LPL, 424 ± 65 cpm/mg; LDLR$^{-/-}$, 404 ± 53 cpm/mg; LDLR$^{-/-}$ Mck-N-LPL, 351 ± 47 cpm/mg). Uptake into other organs was not significantly influenced by LPL expression (**Fig. 5**, upper row). The plasma half-life of ^{125}I-TC LDL was also not significantly affected by the different genotypes, although there was a trend to slower LDL removal on the LDLR$^{-/-}$ background (data not shown). Uptake of LDL into muscle was increased significantly by inactive LPL on the LDLR$^{+/-}$ background [by 77%; LDLR$^{+/-}$, 5.2 ± 1.7 cpm/mg; LDLR$^{+/-}$Mck-N-LPL, 9.2 ± 2.6 cpm/mg ($P < 0.01$)]. However, on the LDLR$^{-/-}$ background, muscle uptake of LDL was not influenced by inactive LPL (LDLR$^{-/-}$, 9.3 ± 1.8 cpm/mg; LDLR$^{-/-}$Mck-N-LPL, 10.5 ± 3.7 cpm/mg). Again, most radioactivity was taken up by the liver, with the uptake into LDLR$^{+/-}$ livers being ~20% higher ($P < 0.05$) compared with that into LDLR$^{-/-}$ livers (LDLR$^{+/-}$, 144 ± 19 cpm/mg; LDLR$^{+/-}$Mck-N-LPL, 145 ± 21 cpm/mg; LDLR$^{-/-}$, 122 ± 12 cpm/mg; LDLR$^{-/-}$ Mck-N-LPL, 129 ± 9.4 cpm/mg). As for chylomicrons, LDL uptake into other organs was not changed significantly by inactive LPL (Fig. 5, lower row). Together, the plasma lipoprotein data and metabolic studies confirmed that LPL-mediated organ uptake of LDL, but not of chylomicrons, is mediated by the LDLR.

Muscle lipid composition

To test whether different expression of inactive LPL and LDLR resulted in intramuscular lipid changes, muscle lipids were extracted and measured. Transgenic expression of inactive LPL on a chow diet resulted in increased muscle TG deposition (~2-fold). On a high-fat diet, a significant increase of TG (4- to 6-fold), cholesterol (3- to 4-fold), and phospholipids (2-fold; data not shown) was found in mice with transgenic expression of inactive LPL in muscle. The presence or absence of the LDLR did not affect these alterations (**Fig. 6**).

TABLE 1. Plasma lipoprotein levels

Genotype	n	TG	Cholesterol	VLDL-TG	VLDL-Cholesterol	LDL-TG	LDL-Cholesterol	HDL-TG	HDL-Cholesterol
Male mice									
LDLR$^{+/-}$	17	73 ± 22	157 ± 16	48 ± 16	23 ± 5.4	42 ± 15	47 ± 10	14 ± 2.3	73 ± 11
LDLR$^{+/-}$Mck-N-LPL	21	77 ± 21	133 ± 19	46 ± 15	22 ± 4.9	32 ± 7.5	36 ± 5.7	13 ± 3.3	64 ± 11
P		NS	<0.005	NS	NS	<0.05	<0.0005	NS	NS
LDLR$^{-/-}$	46	143 ± 44	244 ± 46	81 ± 27	54 ± 18	56 ± 17	118 ± 32	16 ± 3.4	69 ± 12
LDLR$^{-/-}$Mck-N-LPL	43	173 ± 51	245 ± 56	108 ± 49	60 ± 19	61 ± 20	109 ± 29	15 ± 3.1	66 ± 12
P		<0.02	NS	<0.005	NS	NS	NS	NS	NS
Female mice									
LDLR$^{+/-}$	17	82 ± 14	87 ± 16	42 ± 16	19 ± 5.7	23 ± 7.6	39 ± 5.9	13 ± 2.0	45 ± 7
LDLR$^{+/-}$Mck-N-LPL	11	68 ± 14	88 ± 5.1	35 ± 13	18 ± 5.3	20 ± 3.4	34 ± 3.2	12 ± 1.4	48 ± 12
P		NS	NS	NS	NS	NS	<0.01	NS	NS
LDLR$^{-/-}$	36	136 ± 46	213 ± 38	67 ± 26	55 ± 21	37 ± 7.9	117 ± 23	14 ± 3.1	51 ± 10
LDLR$^{-/-}$Mck-N-LPL	28	152 ± 35	215 ± 48	87 ± 28	63 ± 19	38 ± 11	109 ± 30	14 ± 2.7	48 ± 9
P		NS	NS	<0.01	NS	NS	NS	NS	NS

TG, triglyceride. Plasma from individual mice after 6 h of daytime fasting was separated by sequential ultracentrifugation. Mouse genotypes are as follows: LDLR$^{+/-}$, heterozygote low density lipoprotein receptor-deficient; LDLR$^{-/-}$, homozygote low density lipoprotein receptor-deficient; Mck-N-LPL, transgenic expression of inactive LPL in muscle. Data are given as means (mg/dl) ± SD. Student's *t*-test was used to calculate statistical significance on a specific mouse LDLR background.

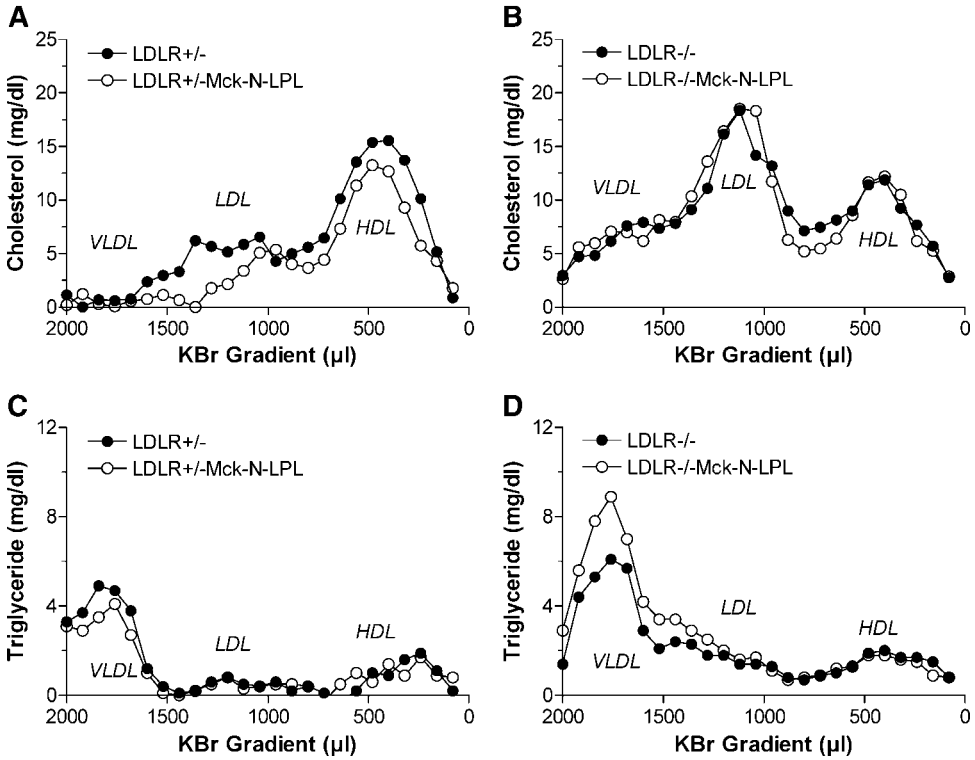

Fig. 4. Plasma lipoprotein profiles by gradient centrifugation from male mice. A, B: Cholesterol profiles. C, D: Triglyceride (TG) profiles. A, C: Heterozygote LDLR-deficient mice ($LDLR^{+/-}$) with and without transgenic inactive LPL (Mck-N-LPL). B, D: Homozygote LDLR-deficient mice ($LDLR^{-/-}$) with and without transgenic inactive LPL. Pooled plasma from five mice (200 μl) was separated on a continuous KBr gradient from 1.21 to 1.0 g/ml (total volume, 2,000 μl). Data from three to four gradients per genotype were used.

Histological studies

Muscles from 5–6 month old mice were examined by histological techniques both after being on a chow diet and after 4 weeks on a Western-type diet. As shown in **Fig. 7** (upper row), $LDLR^{+/-}$ and $LDLR^{-/-}$ mice had normal muscle histology after periodic acid Schiff staining. However, the addition of the Mck-N-LPL transgene on both of these backgrounds resulted in an increased number of muscle fibers with centralized nuclei, pathological periodic acid Schiff staining, and nonspecific signs of muscle damage, such as changed cell shape and increased amounts of connective tissue. Staining for neutral lipids with Oil Red O revealed a large amount of lipid droplets in muscles of $LDLR^{+/-}$Mck-N-LPL and $LDLR^{-/-}$Mck-N-LPL mice (dark red) (Fig. 7, lower row) but not in animals without transgenic expression of inactive LPL. In agreement with the muscle lipid composition, these data show that most of the LPL-mediated muscular lipid uptake occurs without the involvement of the LDLR, presumably from TG-rich particles. LPL-mediated myopathy develops independently of the presence of the LDLR.

DISCUSSION

During the past two decades, several groups have shown in vitro that LPL can augment the binding and uptake of lipoproteins independent of its catalytic activity. This process has been established in various cell types for both TG-rich lipoproteins (3, 12) and LDL (5, 29). In a transgenic mouse model with expression of catalytically inactive LPL, LPL-mediated bridging and uptake of lipo-

TABLE 2. Plasma lipoprotein levels in male mice after 4 weeks on a high-fat diet

Genotype	n	TG	Cholesterol	VLDL-TG	VLDL-Cholesterol	LDL-TG	LDL-Cholesterol	HDL-TG	HDL-Cholesterol
$LDLR^{+/-}$	14	90 ± 8.3	189 ± 18	55 ± 5.6	32 ± 10	15 ± 5.3	84 ± 10	9.9 ± 3.3	90 ± 10
$LDLR^{+/-}$Mck-N-LPL	12	80 ± 6.4	193 ± 29	48 ± 4.9	36 ± 11	13 ± 3.2	74 ± 8.8	8.0 ± 2.6	82 ± 13
P		<0.005	NS	<0.001	NS	NS	0.01	NS	NS
$LDLR^{-/-}$	8	212 ± 43	484 ± 72	158 ± 38	244 ± 78	35 ± 4.5	357 ± 94	32 ± 14	82 ± 10
$LDLR^{-/-}$Mck-N-LPL	9	273 ± 59	523 ± 63	213 ± 75	287 ± 123	38 ± 7.3	353 ± 118	37 ± 16	93 ± 9.8
P		<0.05	NS	NS	NS	NS	NS	NS	NS

For details, see Table 1 legend. Data are given as means (mg/dl) ± SD.

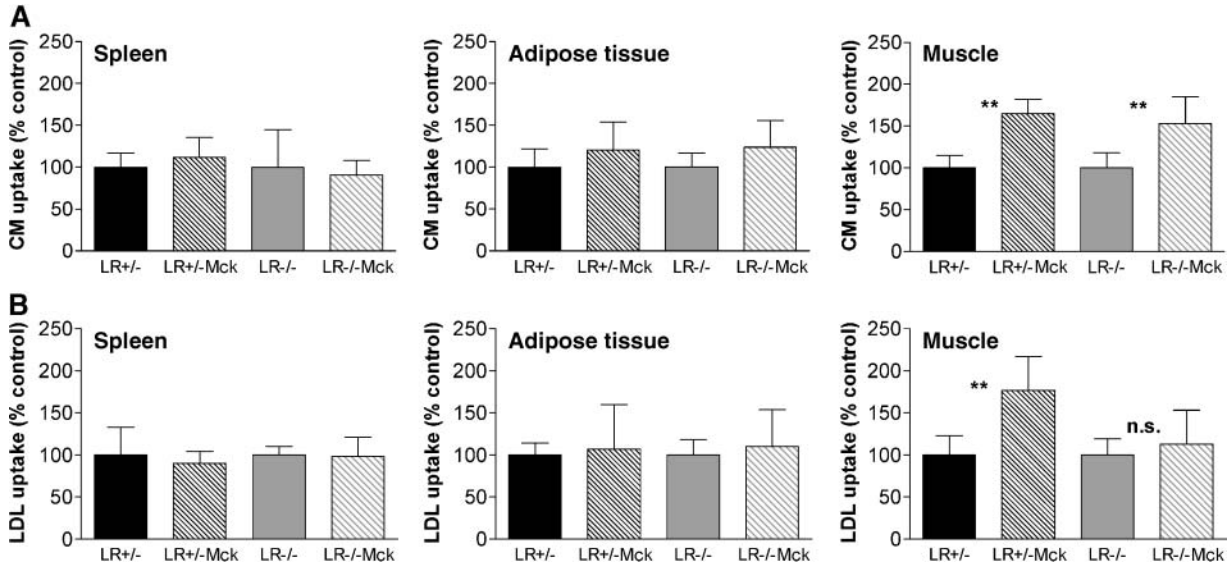

Fig. 5. Organ uptake of lipoproteins in mice. Upper panels: Uptake of chylomicrons (CM). ^{125}I-tyramine cellobiose-labeled chylomicrons were injected into six to seven mice per genotype. Organs were harvested after 60 min. Lower panels: Uptake of LDL. ^{125}I-tyramine cellobiose-labeled LDL was injected into seven to eight mice per genotype. Organs were harvested after 20 h. Transgenic inactive LPL (Mck-N-LPL) increased the uptake of chylomicrons into muscle both on the heterozygote ($^{+/-}$) and on the homozygote ($^{-/-}$) LDLR-deficient background. Muscle uptake of LDL was enhanced only on the heterozygote LDLR-deficient background. Other organs were not significantly affected. Uptake into each organ on the respective LDLR background without the expression of inactive LPL was set to 100%. Values shown are means ± SD. ** $P < 0.001$ versus the equal LDLR genotype. n.s., not significant.

proteins was proven in vivo (6). However, this metabolic pathway required at least some active LPL in the same organ (7). Binding of LPL to lipoproteins could occur either by hydrophobic interactions (30, 31) or directly via apolipoprotein B (32, 33). At the cellular site, lipoprotein receptors (2) and proteoglycans (3, 34) have been postulated to be involved in LPL binding. For VLDLR (9, 10, 35), gp330 (11), and LRP1 (12, 13), binding of LPL to the receptors was clearly demonstrated in vitro. In contrast, there are conflicting data for the LDLR. Some scientists did not find evidence of LPL binding to the LDLR (5, 12), whereas others reported that LPL can enhance the cellular uptake of TG-rich lipoproteins by interactions with LDLR (4, 14), suggesting direct LPL-LDLR interaction.

The present study was performed to clarify the role of LDLR during LPL-mediated uptake of lipoproteins. Lipoprotein uptake studies with primary aortic endothelial cells from wild-type and LDLR$^{-/-}$ mice showed that for LPL-mediated uptake of LDL, but not of chylomicron remnants, the presence of LDLR was obligatory. Thus, our results confirmed previously published data (4, 14). The advantage of using a primary endothelial cell approach with inbred mouse lines was that, except for LDLR status,

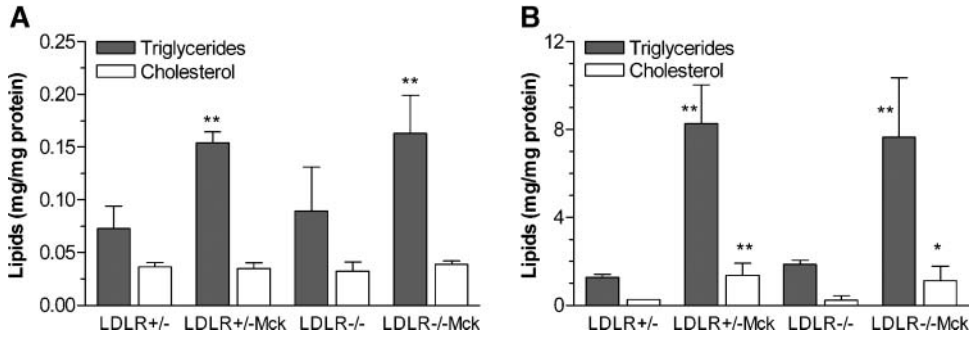

Fig. 6. Muscle lipid content of mice on chow (left panel) and on a Western-type diet (right panel). Lipids of femoral and hamstring muscles of 5-6 month old male mice were extracted, and total cholesterol and TGs were measured using commercial kits. On the chow diet, TGs were increased, and on the Western diet, both TGs and cholesterol were increased by catalytically inactive LPL independent of the presence of the LDLR. Mouse lines are as described for Fig. 5. Values shown are means ± SD. Student's *t*-test was used to compare the respective concentrations on the corresponding LDL receptor backgrounds. * $P < 0.01$, ** $P < 0.001$ for TG and cholesterol, respectively, compared with the same LDLR genotype. n.s., not significant.

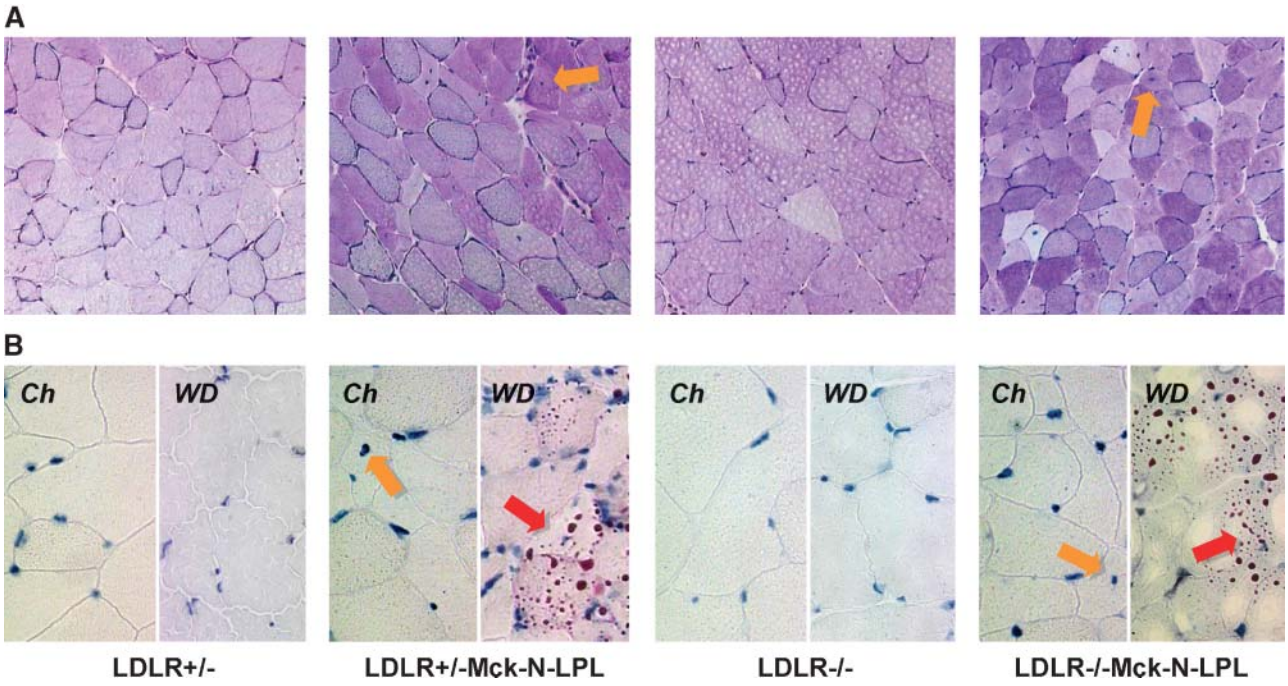

Fig. 7. Muscle histology. Femoral muscles from 5–6 month old male mice were stained using routine techniques. Upper row: Periodic acid Schiff staining (113×). Lower row: Oil Red O staining (400×). Ch, on a chow diet; WD, after 4 weeks on a Western-type diet. Overexpression of inactive LPL in muscle (Mck-N-LPL) led to a myopathy with inhomogeneous muscle fibers and centralized nuclei (orange arrows). On the Western diet, inactive LPL also resulted in an accumulation of lipid droplets (red arrows). All effects were independent of the presence of the LDLR.

genetically identical cells could be used. In addition, these data might reflect the roles of both LPL and LDLR in the endothelial uptake of lipoproteins (36). Because, to our knowledge, there are no HSPG-deficient endothelial cells, the function of HSPG in this process was evaluated using normal and HSPG-deficient CHO cells. Even a marked upregulation of the LDLR in the cells by LPDS/lovastatin was not able to compensate for the lack of HSPG. This confirms the importance of HSPG for LPL-mediated uptake of LDL, as suggested by others (15, 37).

To prove the physiological relevance of these results, animal studies were performed using a mouse model with expression of catalytically inactive LPL both on the heterozygote and on the homozygote LDLR-deficient background. Inactive LPL was able to reduce plasma LDL on the heterozygote, but not on the homozygote, LDLR-deficient background both on chow and on a high-fat Western-type diet. On the LDLR-deficient background, transgenic expression of inactive LPL led to increased VLDL-TG levels. Previous studies (6) have shown that inactive LPL on the wild-type background accelerates plasma TG hydrolysis, resulting in lower plasma TG. It is not clear what reverses this effect in the absence of the LDLR. Increased amounts of apolipoprotein E on non-HDL lipoproteins in $LDLR^{-/-}$ mice may inhibit LPL-mediated lipolysis, as has been suggested (38). Excess LDL could somehow interfere with plasma TG lipolysis. In addition, inactive LPL could increase hepatic remnant uptake by binding to LRP1, leading to greater VLDL production (8). This could bring LPL-mediated lipolysis closer to saturation, and a defect in LPL, perhaps attributable to the dimerization of inactive and active LPL or inactive LPL displacing active LPL from the vessel wall, may become apparent.

Metabolic studies showed that LPL facilitates the lipoprotein uptake of both chylomicrons and LDL into muscle. Because LDL does not have significant amounts of hydrolyzable TG, it seems to be small enough to cross the endothelium in vivo, and an increased endothelial permeability, as suggested previously (39), is not necessary. As found in cell culture studies, an increased uptake of LDL was only possible in the presence of the LDLR. This demonstrated the necessity of this receptor for the LPL-augmented endothelial transcytosis and uptake of apolipoprotein B-100-containing lipoproteins in vivo. However, our experiments do not prove a direct interaction between LPL and the LDLR. LPL could also concentrate LDL in the vicinity of the LDLR, resulting in increased cellular uptake, as suggested by colocalization data of LDL and LDLR in endothelial cells (Fig. 1C) (4). On the other hand, for apolipoprotein E/apolipoprotein B-48-containing particles, such as chylomicrons and VLDLs, LDLR deficiency did not lead to a reduction of LPL-facilitated lipoprotein transport into muscle. Therefore, the LDLR is not likely to be involved in the transport of these lipoproteins. VLDLR, other receptors, or HSPG may mediate the LPL-augmented uptake of TG-rich lipoproteins, as suggested previously (2, 3, 35).

On a high-fat diet, LPL expression markedly increased the muscle storage of TG, phospholipids, and cholesterol, which was not influenced by the presence or absence of LDLR. In concert with the metabolic data, this illustrates that the importance of the uptake of LDL for compound and energy supply in muscle is rather low compared with that of the LPL-mediated uptake of fatty acids, phospholipids, and cholesteryl esters from TG-rich particles. Muscle histology from our mouse lines showed an LPL-mediated myopathy independent of the presence of LDLR. A myopathy as the result of transgenic muscle LPL expression was first shown by Levak-Frank et al. (40). Initially, it was attributed to the mitochondrial toxicity of fatty acids shuttled into muscle by LPL-mediated TG hydrolysis. Later, direct LPL muscle toxicity was ruled out (41). Our data are in agreement with previous experiments showing that muscle damage was most likely attributable to LPL-mediated increased selective cholesteryl ester uptake (7). Although it presumably does not proceed via the LDLR, the precise mechanism of this pathway is not clear. LPL-mediated approximation of lipoproteins to the cell surface via binding to HSPG could play a role (42, 43).

In summary, it was shown in vitro and in vivo that the LPL-mediated uptake of LDL into endothelial cells proceeds via the LDLR. In addition, HSPGs are obligatory for this process. It is likely that LPL bound to HSPG concentrates LDL in the vicinity of the LDLR, which then performs the actual LDL uptake.

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