

Effects of lipoprotein lipase and statins on cholesterol uptake into heart and skeletal muscle^S

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Abstract Regulation of cholesterol metabolism in cultured cells and in the liver is dependent on actions of the LDL receptor. However, nonhepatic tissues have multiple pathways of cholesterol uptake. One possible pathway is mediated by LPL, an enzyme that primarily hydrolyzes plasma triglyceride into fatty acids. In this study, LDL uptake and tissue cholesterol levels in heart and skeletal muscle of wild-type and transgenic mice with alterations in LPL expression were assessed. Overexpression of a myocyte-anchored form of LPL in heart muscle led to increased uptake of LDL and greater heart cholesterol levels. Loss of LDL receptors did not alter LDL uptake into heart or skeletal muscle. To induce LDL receptors, mice were treated with simvastatin. Statin treatment increased LDL receptor expression and LDL uptake by liver and skeletal muscle but not heart muscle. Plasma creatinine phosphokinase as well as muscle mitochondria, cholesterol, and lipid droplet levels were increased in statin-treated mice overexpressing LPL in skeletal muscle. **FF** Thus, pathways affecting cholesterol balance in heart and skeletal muscle differ.—Yokoyama, M., T. Seo, T. Park, H. Yagyu, Y. Hu, N. H. Son, A. S. Augustus, R. K. Vikramadithyan, R. Ramakrishnan, L. K. Pulawa, R. H. Eckel, and I. J. Goldberg. **Effects of lipoprotein lipase and statins on cholesterol uptake into heart and skeletal muscle.** *J. Lipid Res.* 2007. 48: 646–655.

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Heart and skeletal muscle are among the lowest cholesterol biosynthetic tissues of the body (1) and, as for many tissues, circulating lipoproteins probably supply muscle cholesterol needs. Although this could occur via LDL receptor uptake, a curious aspect of the regulation of the fibroblast LDL receptor is that the receptor is half maximally saturated by subphysiologic levels (30 $\mu\text{g}/\text{ml}$) of LDL cholesterol (2). Thus, if the fibroblast is represen-

tative of muscles, the LDL receptor should be down-regulated and an alternative process must lead to the acquisition of plasma LDL. Such a conclusion is consistent with studies showing that heart and skeletal muscle take up very little LDL from the circulation (3, 4).

There are likely to be other pathways mediating cellular cholesterol uptake by heart and skeletal muscle. These pathways could involve the uptake of cholesterol from lipoproteins other than LDL or could include the selective uptake of LDL cholesterol (i.e., acquisition of lipid exclusive of whole particles). In the case of HDL, selective uptake of lipoprotein lipids occurs via scavenger receptors (5). The selective uptake of cholesterol from LDL can be mediated by LPL (6, 7), the primary enzyme responsible for intravascular hydrolysis of triglyceride (TG). This process might be especially important in skeletal and heart muscle that have robust LPL expression.

Cells must modulate cholesterol content to prevent lipid intoxication. The liver eliminates excess cholesterol into the bile; adipose tissue can store excess cholesterol within lipid droplets. Muscles might need to more finely regulate cholesterol uptake. Inappropriate upregulation of LDL receptors leading to excess cellular cholesterol could be pathologic (8). Similarly, receptor-mediated increased muscle uptake of lipoproteins could lead to potentially toxic levels of phospholipids, TGs, and fatty acids.

Previously, we created mice that develop a dilated cardiomyopathy and excess cholesterol in the heart (9). These mice express a transgene for a cardiomyocyte-anchored form of human LPL; they are denoted LPL^{GPI} because the

Abbreviations: CPK, creatine phosphokinase; HMG-CoA-R, hydroxymethyl glutaryl coenzyme A reductase; *Ldlr*^{-/-}, low density lipoprotein receptor knockout; LPL^{GPI}, glycosylphosphatidylinositol-anchored lipoprotein lipase; MCK, muscle creatinine kinase; *Srebp2*, sterol-regulatory element binding protein 2; TC, tyramine cellobiose; TG, triglyceride.

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LPL has a glycosylphosphatidyl-inositol anchor. LPL is the rate-limiting enzyme responsible for plasma TG metabolism but not LDL cholesterol metabolism. In this study, we assessed whether the expression of LPL and the LPL^{GPI} transgene altered LDL uptake into heart and skeletal muscles. The LPL^{GPI} transgene increased LDL uptake into the heart, but this was exclusive of the presence of LDL receptors. When mice were treated with high doses of a hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA-R) inhibitor (statin), LDL receptor expression increased in skeletal muscle but not in heart muscle. In the presence of excess muscle LPL, statin treatment caused muscle toxicity.

METHODS

Materials

Simvastatin as Zocor® (Merck, Whitehouse Station, NJ) was obtained in tablet form. The tablets were powdered and mixed with normal chow diet that contained 5.0% (w/w) fat (Research Diets, Inc., New Brunswick, NJ). The final concentration of simvastatin in the mixtures was 0.1% of the diet.

Animals and experimental treatments

LPL^{GPI} transgenic mice (9) and muscle creatinine kinase (MCK)-LPL mice (10) have been described. Low density lipoprotein receptor knockout (*Ldlr*^{-/-}) mice on the C57BL/6 background were purchased from Jackson Laboratory, and the MCK-LPL and LPL^{GPI} mice were bred for more than six generations onto this background. MCK-LPL mice were cross-bred with *Ldlr*^{-/-} mice to obtain MCK-LPL/*Ldlr*^{-/-} mice. As was noted on the wild-type background (10), the MCK-LPL transgene increased muscle TG (from 1 to 6 µg/mg) and fatty acids (from 0.25 to 0.4 nmol/mg) but led to smaller differences in tissue cholesterol in a small number of *Ldlr*^{-/-} mice. All mice were housed in a temperature-controlled (25°C) facility with a 12 h light/dark cycle.

For statin diet treatment, 10–12 week old male wild-type C57BL/6, *Ldlr*^{-/-}, MCK-LPL, and MCK-LPL/*Ldlr*^{-/-} mice were fed for 2 weeks with either the control chow diet or a diet that contained 0.1% simvastatin. Blood from fasted (24 h) mice was collected from the retro-orbital plexus into tubes containing EDTA. Enzymatic kits were used to determine plasma TG and total cholesterol (Thermo Electron Co.), free fatty acid (Wako Chemicals USA, Richmond, VA), glucose (Lifescan, Inc., Milpitas, CA), and creatine phosphokinase (CPK; Olympus Diagnostica GmbH) levels, all in duplicate (11).

Northern blot analysis

Total RNA (10 µg) was isolated from liver, heart, and skeletal muscle using TRIzol reagent (Invitrogen, Carlsbad, CA), subjected to electrophoresis on a 1% agarose gel containing formamide, and transferred to nylon filters (Hybond N; Amersham, Piscataway, NJ). Northern blot analyses were performed using the radiolabeled cDNA probes for atrial natriuretic factor, brain natriuretic protein, LDL receptor, and HMG-CoA-R. The data were normalized to the expression of GAPDH. The blots were quantified using densitometric scanning.

Real-time reverse transcription PCR

Sterol-regulatory element binding protein 2 (*Srebp2*), HMG-CoA-R, LDL receptor (*Ldlr*), cyclophilin, and β-actin mRNA levels were determined by real-time reverse transcription PCR. RNA integrity was checked by ethidium bromide staining after

electrophoresis. cDNA was produced from total RNA (1 µg) via reverse transcription using SuperScript reverse transcriptase (Invitrogen) in a 20 µl reaction volume containing 1 × SuperScript buffer (Invitrogen), 1 mM deoxynucleoside triphosphate, 20 µg of random hexamers, 10 mM dithiothreitol, and 20 units of RNase inhibitors. After 60 min at 55°C incubation, the reaction was stopped (10 min at 85°C). *Ldlr*, *Srebp2*, HMG-CoA-R, cyclophilin, and β-actin were amplified by PCR using Syber PCR master mix (Applied Biosystems) and the following primer sequences: *Ldlr* (forward, 5'-GAAGTCGACACTGTACTGACCAC-CACC-3'; reverse, 5'-CTCCTCATTCCCTCTGCCAGCCAT-3'); *Srebp2* (forward, 5'-AGGTAATAACCCCGTATATCC-3'; reverse, 5'-GATACCACGATTGTTTTGG-3'); HMG-CoA-R (forward, 5'-CGC-AACCTCTATATCCGT-3'; reverse, 5'-GTAGCCGCTATGCTC-3'); cyclophilin (forward, 5'-ATGTGCCAGGGTGGTGACTT-3'; reverse, 5'-GCCATCCAGCCATTCACTCT-3'); and β-actin (forward, 5'-TG-AAGGTGACGTTGACA-3'; reverse, 5'-TAGAAGCACTTGCGGT-GCAG-3'). Optimized PCR consisted of 40 cycles of amplification at 95°C for 15 s followed by amplification at 60°C for 1 min. Significant PCR fluorescent signals were normalized for each sample to PCR fluorescent signals obtained from two independent controls, cyclophilin and β-actin genes.

Electron microscopy

Electron microscopy was performed on skeletal muscle (quadriceps femoris) fixed with 2.5% glutaraldehyde in 0.1 M Sorensen's buffer (0.2 M monobasic phosphate and 0.2 M dibasic phosphate, 1:4, v/v, pH 7.2), postfixed in osmium tetroxide, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM-1200ExII electron microscope (JEOL Ltd., Tokyo, Japan).

Lipoprotein uptake studies

LDLs were isolated from *Ldlr*^{-/-} mice (n = 25) by sequential ultracentrifugation at 1.025 < d < 1.063 for 30 h. Immediately after isolation, LDLs were labeled with ¹²⁵I-labeled-tyramine cellobiose (TC) (11). ¹²⁵I-TC was prepared using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Iodogen; Pierce Biotechnology, Inc., Rockford, IL) and linked to LDL by activating the iodinated TC with cyanuric chloride as described previously (11). Labeled LDLs were dialyzed extensively against saline containing 1 mg/ml EDTA, sterilized by filtration through an Acrodisc (0.45 µm), stored at 4°C, and used within 1 week. One hundred micrograms of ¹²⁵I-TC-LDL (120 cpm/ng protein) was injected via saphenous vein. The mice remained fasting during the 20 h study. At the end of the experiment, the mice were anesthetized, exsanguinated, and then perfused with 10 ml of PBS containing 1 mg/ml EDTA. Isolated organs were quickly rinsed with ice-cold PBS and weighed, and radioiodine was quantified by an automatic γ counter (WALLAC 1470 wizard; Perkin-Elmer). To normalize for plasma pool size and the slower loss of labeled LDL from the circulation in *Ldlr*^{-/-} mice, ¹²⁵I-TC-LDL uptake was corrected using the plasma decay curves.

Tissue lipid analysis

Heart, liver, and skeletal muscle were rapidly removed and homogenized in ice-cold 1 M NaCl buffer containing protease inhibitors to prevent TG hydrolysis. Lipids were extracted from these tissues (50 mg) according to methods modified from that of Folch, Lees, and Sloane Stanley (12). Briefly, snap-frozen tissues were homogenized and extracted twice with chloroform-methanol (2:1, v/v) solution. The organic phase was dried under nitrogen gas and resolubilized in chloroform. An aliquot of the Folch extraction was resuspended in an aqueous solution containing 2% Triton X-100. Total and free cholesterol, TG, and FFA levels were determined with enzymatic kits as described (11).

Statistical analysis

Student's *t*-tests of group means were used to compare groups with statistically significant differences at $P < 0.05$. Because of the variability of uptake, initial studies of TC-LDL uptake performed in statin-treated wild-type mice did not reach statistical significance; the experiment was repeated and the data were merged. ANOVA was used for comparisons among different groups and to evaluate potential interactions between different groups.

RESULTS

Effects of LDL receptor deficiency on cardiac lipids and LDL uptake in LPL^{GPI} mice

Mice with or without the LPL^{GPI} transgene had identical plasma clearance of ¹²⁵I-TC-LDL (Fig. 1A). As expected, liver uptake of LDL was not affected by the LPL^{GPI} trans-

gene (Fig. 1B). However, the LPL^{GPI} transgene doubled LDL uptake into the heart (Fig. 1C).

LPL is not normally considered a major mediator of cellular cholesterol uptake. However, LPL can concentrate lipoproteins on the cell surface and increase their proximity to lipoprotein receptors. To test whether this process was occurring, we crossed the LPL^{GPI} transgene onto the *Ldlr*^{-/-} background. *Ldlr*^{-/-} mice had 3.6-fold greater levels of plasma cholesterol than did wild-type mice (Table 1). The LPL^{GPI} transgene did not alter plasma lipids when crossed onto the wild-type or *Ldlr*^{-/-} background. However, *Ldlr*^{-/-}/LPL^{GPI} hearts had 70% more cardiac cholesterol and 20% more FFA than did *Ldlr*^{-/-} mice (Table 2). *Ldlr*^{-/-} mice, as expected, had delayed plasma LDL turnover (Fig. 1A) and reduced liver LDL uptake (Fig. 1B). However, loss of LDL receptors did not alter cardiac LDL uptake. Hearts from *Ldlr*^{-/-}/LPL^{GPI} mice still

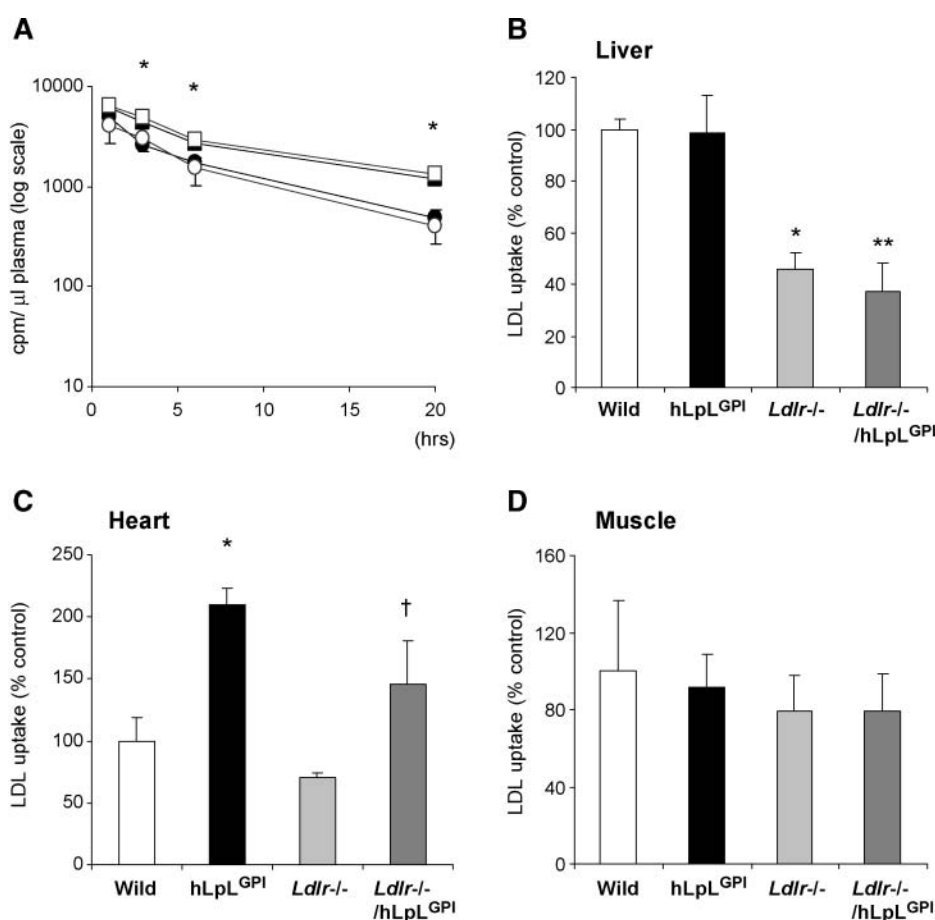


Fig. 1. LDL kinetic studies in wild-type, low density lipoprotein receptor knockout (*Ldlr*^{-/-}), glycosylphosphatidylinositol-anchored lipoprotein lipase (LPL^{GPI}), and *Ldlr*^{-/-}/LPL^{GPI} mice. A: Plasma clearance of ¹²⁵I-tyramine cellobiose (TC)-LDL in wild-type, LPL^{GPI}, *Ldlr*^{-/-}, and *Ldlr*^{-/-}/LPL^{GPI} mice. Male mice (10–12 weeks old) were used for these and subsequent studies with an additional 2 week feeding period as described in Methods. ¹²⁵I-TC-LDL was injected into wild-type (open circles; *n* = 4), LPL^{GPI} (closed circles; *n* = 5), *Ldlr*^{-/-} (open squares; *n* = 4), and *Ldlr*^{-/-}/LPL^{GPI} (closed squares; *n* = 4) animals. At the indicated times, blood was collected. Values are expressed as means ± SD. * $P < 0.05$ versus *Ldlr*^{-/-} background. B–D: Tissue ¹²⁵I-TC-LDL uptake into liver (B), heart (C), and skeletal muscle (D). Twenty hours after injection, the mice were perfused with phosphate-buffered saline containing 1 mg/ml EDTA, and the indicated tissues were removed, washed, and counted. The data are shown as percentages of wild-type (Wild) mice (cpm/g tissues/initial plasma activity). Values are expressed as means ± SD. * $P < 0.05$ versus wild-type mice; ** $P < 0.05$ versus LPL^{GPI} mice; † $P < 0.05$ versus *Ldlr*^{-/-} mice.

TABLE 1. Plasma lipids in LPL^{GPI} and *Ldlr*^{-/-}/LPL^{GPI} mice

Mouse	Cholesterol	TG
Wild type	66.2 ± 4.4	66.8 ± 18.9
<i>Ldlr</i> ^{-/-}	242.1 ± 15.2 ^a	84.9 ± 21.5
LPL ^{GPI}	66.5 ± 3.7	63.6 ± 17.5
<i>Ldlr</i> ^{-/-} /LPL ^{GPI}	241.2 ± 10.3 ^b	83.5 ± 25.8

Ldlr^{-/-}, low density lipoprotein receptor knockout; LPL^{GPI}, glycosylphosphatidylinositol-anchored lipoprotein lipase; TG, triglyceride. Blood was taken from 24 h fasted mice. Values are expressed as means ± SD.

^a *P* < 0.05 versus the wild type.

^b *P* < 0.05 versus LPL^{GPI}.

acquired twice as much TC-labeled LDL as did *Ldlr*^{-/-} hearts (Fig. 1C).

The heart failure markers atrial natriuretic factor and brain natriuretic protein were not reduced in *Ldlr*^{-/-}/LPL^{GPI} hearts compared with LPL^{GPI} hearts. Moreover, loss of the LDL receptor did not affect the mortality of LPL^{GPI} mice (Fig. 2).

There are pathways other than via the LDL receptor whereby LPL could increase the cellular uptake of LDL. LPL allows lipoproteins to associate with cell membrane proteoglycans, and the lipoproteins can be internalized along with recycling of the cell membrane or with other members of the LDL receptor superfamily (13). Our data demonstrate that cardiomyocyte cell surface LPL did not use the LDL receptor to augment LDL uptake.

Effects of loss of LDL receptor on skeletal muscle uptake of TC-LDL

It is possible that the metabolism of cholesterol in the heart differs from cholesterol metabolism in other muscles. For this reason, we also determined the role of the LDL receptor in LDL uptake by skeletal muscle. As for the heart, loss of LDL receptors had little effect on LDL uptake by skeletal muscle (Fig. 1D).

Effects of statins on muscle LDL uptake

We tested whether increasing LDL receptors in the skeletal muscle would alter LDL cholesterol uptake. HMG-CoA-R inhibitors (statins) block the rate-limiting enzyme for cholesterol biosynthesis. By initially reducing cellular cholesterol, they lead to the activation of SREBP2 and

TABLE 2. Heart lipids in LPL^{GPI} and *Ldlr*^{-/-}/LPL^{GPI} mice

Mouse	Total Cholesterol	TG	FFA
	μg/mg heart		nmol/mg heart
Wild type	1.08 ± 0.24	5.15 ± 0.32	1.32 ± 0.11
<i>Ldlr</i> ^{-/-}	1.89 ± 0.22	4.90 ± 0.82	1.25 ± 0.14
LPL ^{GPI}	3.45 ± 0.18 ^a	5.70 ± 0.55	1.62 ± 0.10 ^a
<i>Ldlr</i> ^{-/-} /LPL ^{GPI}	3.22 ± 0.15 ^b	5.54 ± 0.78	1.59 ± 0.13 ^b

Cardiac lipids were measured with enzymatic tests. Values shown are means ± SD.

^a *P* < 0.05 versus the wild type.

^b *P* < 0.05 versus *Ldlr*^{-/-}.

increase the expression of genes within the cholesterol biosynthetic pathway and the expression of the LDL receptor (14). This latter action allows greater LDL uptake by the liver and decreases circulating concentrations of LDL (15).

We treated both wild-type and MCK-LPL mice with high doses of simvastatin. As shown by others, average plasma cholesterol was not reduced significantly by statin in wild-type mice (Table 3). Liver uptake of tracer TC-LDL was increased (Fig. 3A). Heart LDL uptake was not altered by the statin (Fig. 3B). In contrast, skeletal muscle LDL uptake increased in wild-type mice by ~30% from untreated controls (Fig. 3C). Although statins block the rate-limiting enzyme required for cholesterol biosynthesis, muscle cholesterol was not decreased by this therapy (Fig. 3D). These data are consistent with studies showing that muscles have little de novo cholesterol biosynthesis (1).

Statin treatment increased LDL uptake in MCK-LPL mice

Mice with gross overexpression of LPL in muscle via the MCK promoter develop a myopathy that is thought to reflect increased fatty acid flux into the tissue (10). Animals with less flagrant LPL overexpression appear normal, although the muscles of these mice accumulate more TG than do wild-type mice and have some degree of insulin resistance (16, 17). Selective uptake of LDL cholesterol (i.e., uptake of cholesterol without the apolipoprotein B representative of holo-LDL) is increased in skeletal muscle of MCK-LPL mice; however, holoparticle uptake is not (6). In our studies, the presence of the MCK-LPL transgene alone did not alter plasma turnover of TC-LDL. A statistically significant increase in LDL particle uptake into skeletal muscle was also not found (Fig. 3C). Statin treatment increased LDL uptake in skeletal but not heart muscle of MCK-LPL mice (Fig. 3B, C). Mice expressing the MCK-LPL transgene had increased muscle free and total cholesterol levels (Fig. 3D). Statin treatment further increased muscle cholesterol content in these mice. Muscle LPL activity was not altered by statin treatment (data not shown).

Regulation of LDL receptors in heart and skeletal muscle

The major pharmacologic effect of statins in humans is an increase in the clearance of LDL from the blood (15). We explored whether statin therapy induced LDL receptors in the liver, as expected, and in muscles of the treated mice. Both *Ldlr* and HMG-CoA-R mRNA levels were increased in the liver. Statin treatment also increased skeletal muscle *Ldlr* expression by 2-fold; HMG-CoA-R mRNA increased by 1.5- and 1.7-fold in wild-type and MCK-LPL mice, respectively (Fig. 4A and quantification shown in supplementary Fig. 1). In contrast, expression of these two genes in hearts was unaltered.

Skeletal muscle changes with statins

Although statins were developed as cholesterol biosynthetic inhibitors, our data showed that statin treatment could actually increase muscle cholesterol in some strains

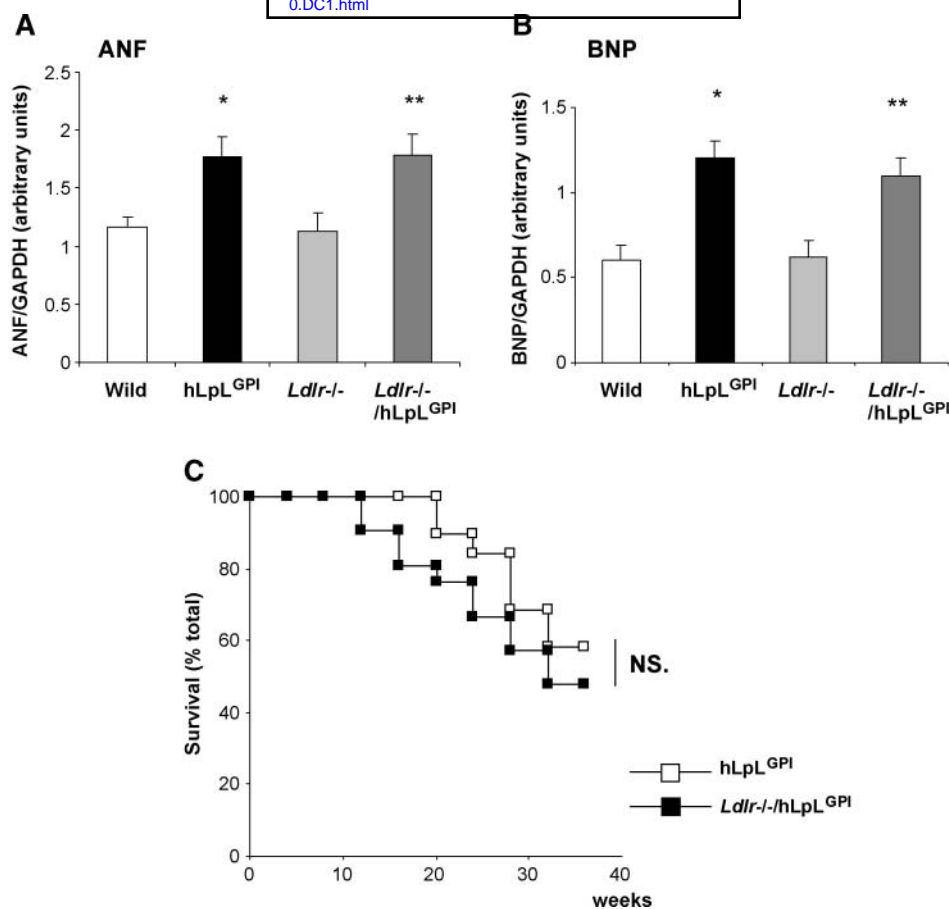


Fig. 2. Effects of the loss of LDL receptors on heart failure markers and mortality of LPL^{GPI} mice. Atrial natriuretic factor (ANF; A) and brain natriuretic protein (BNP; B) mRNA levels in ventricular muscle of 3–4 month old male wild-type (Wild), *Ldlr*^{-/-}, LPL^{GPI}, and *Ldlr*^{-/-}/LPL^{GPI} mice were measured by RT-PCR (n = 3). The survival of LPL^{GPI} and *Ldlr*^{-/-}/LPL^{GPI} mice is shown in C. Values are expressed as means ± SD. * *P* < 0.05 versus control; ** *P* < 0.05 versus *Ldlr*^{-/-}.

of mice. We determined whether greater tissue cholesterol had altered the muscles of statin-treated mice. Control mice showed some increase in mitochondria after statin treatment (Fig. 4B). Mitochondrial accumulation in the subsarcolemmal region was increased in nontreated MCK-LPL muscle; however, the increase was greatest in muscle from statin-treated MCK-LPL mice (Fig. 4B). Statin-treated MCK-LPL mice also had more and larger lipid droplets around the perinuclear region than those in muscles of untreated mice and statin-treated wild-type mice (Fig. 4B, C). Further evidence of muscle damage was obtained by measuring plasma CPK, which increased by 3.8-fold in statin-treated MCK-LPL mice (Fig. 4D). Every mouse in

this group had a greater plasma CPK level than did untreated or nontransgenic mice.

Statin effects in MCK-LPL/*Ldlr*^{-/-} mice

If statin-induced muscle toxicity results from excess lipoprotein uptake via stimulation of the LDL receptor pathway, then genetic loss of the LDL receptor should prevent this effect. To test this hypothesis, we treated MCK-LPL/*Ldlr*^{-/-} mice with statin. Loss of the LDL receptor prevented the statin-mediated changes; muscle LDL uptake, muscle cholesterol levels, and plasma CPK increase were unaltered (see supplementary Fig. IIA–C). Although it is most logical to explain the lack of toxicity in the *Ldlr*^{-/-}

TABLE 3. Plasma lipids in wild-type and MCK-LPL mice with statin treatment

Mouse	Cholesterol	TG	FFA	Glucose
Wild type	102.9 ± 17.8	64.6 ± 12.2	0.93 ± 0.18	100.5 ± 22.9
Wild type + statin	92.6 ± 23.3	53.9 ± 4.6	0.75 ± 0.10	120.5 ± 26.0
MCK	97.1 ± 22.7	48.1 ± 7.1	0.69 ± 0.15	92.0 ± 16.0
MCK + statin	67.9 ± 8.9 ^a	38.5 ± 3.7 ^a	0.64 ± 0.05 ^a	122.0 ± 39.6

MCK, muscle creatinine kinase. Blood was taken from 24 h fasted mice. Values are expressed as means ± SD. ^aSignificant differences for with versus without statin treatment (*P* < 0.05).

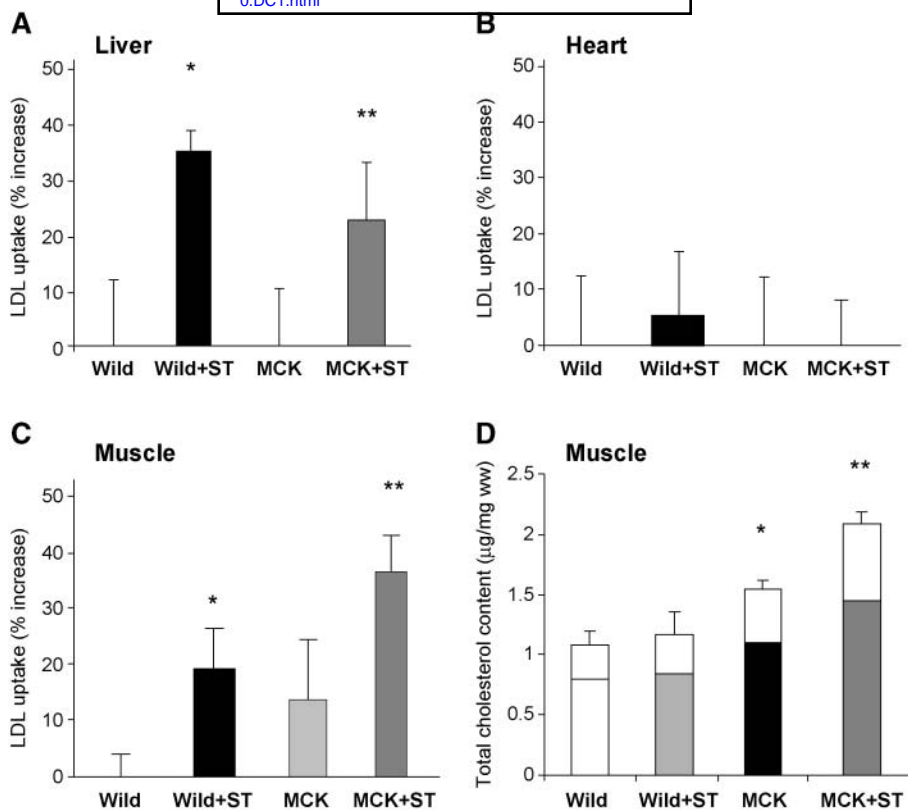


Fig. 3. Effects of statin treatment on LDL kinetics and tissue uptake in wild-type and muscle creatinine kinase (MCK)-LPL mice. A–C: Tissue ^{125}I -TC-LDL uptake into liver (A), heart (B), and skeletal muscle (C). ^{125}I -TC-LDL was injected into male wild-type mice (Wild; $n = 11$), wild-type mice treated with statin (Wild+ST; $n = 12$), MCK-LPL mice (MCK; $n = 7$), and MCK-LPL mice treated with statin (MCK+ST; $n = 8$). Twenty hours after injection, the mice were perfused with phosphate-buffered saline containing 1 mg/ml EDTA, and the indicated tissues were removed, washed, and counted. The data are shown as percentage increase in uptake compared with tissues of untreated wild-type mice (cpm/g tissues/initial plasma activity). Values are expressed as means \pm SD. * $P < 0.05$ versus wild-type mice; ** $P < 0.05$ versus MCK-LPL mice. D: Muscle cholesterol contents were measured with enzymatic tests. Free cholesterol is shown in the shaded areas, and ester is shown in the open areas. Values are expressed as means \pm SD. ww, muscle wet weight. * $P < 0.05$ versus wild-type mice; ** $P < 0.05$ versus MCK-LPL mice.

mice as a result of the drug's failure to induce excess lipoprotein uptake, these mice have other metabolic alterations, such as hyperlipidemia, that might, by unknown mechanisms, also have prevented the statin myopathy.

DISCUSSION

We studied the importance of LPL and its regulation on LDL uptake in cardiac and skeletal muscle using animals with tissue-specific expression of LPL. Our data show the following. 1) LPL on the cardiomyocyte surface increased heart uptake of LDL. 2) Under control situations, the LDL receptor had little effect on LDL uptake into muscle or heart. 3) With high doses of statin, LDL receptors were upregulated in the skeletal muscle and this was associated with increased LDL uptake. 4) Mice that had increased expression of muscle LPL had more muscle cholesterol, lipid droplets, and mitochondria. Plasma CPK was increased in these mice, indicating muscle damage. 5) This effect was not seen when LDL receptors were deleted.

6) Heart uptake of LDL was unaffected by the loss of LDL receptors or statin treatment.

We had previously created a mouse model of lipotoxic cardiomyopathy that was associated with increased uptake of plasma lipoproteins (9). The most significantly increased lipid was cholesterol. Thus, we tested whether the transgene expressing cardiomyocyte-anchored LPL increased heart uptake of LDL and found that it did. This suggested that LPL on the cardiomyocyte surface could be an important mediator of heart cholesterol uptake. Surprisingly, the cardiomyopathy was not improved by crossing the LPL^{GPI} mice with *Ldlr*^{-/-} animals (i.e., loss of LDL receptors did not reduce the uptake of plasma LDL). Therefore, heart uptake of LDL in LPL^{GPI} mice was exclusive of LDL receptors.

The heart is a major site of plasma lipoprotein metabolism; loss of only cardiac LPL leads to fasting and postprandial hyperlipidemia in mice (18), and cardiac-specific LPL expression is sufficient to maintain normal plasma lipid levels (19). Normally, hearts acquire very little cholesterol from LDL. The heart does acquire cholesterol

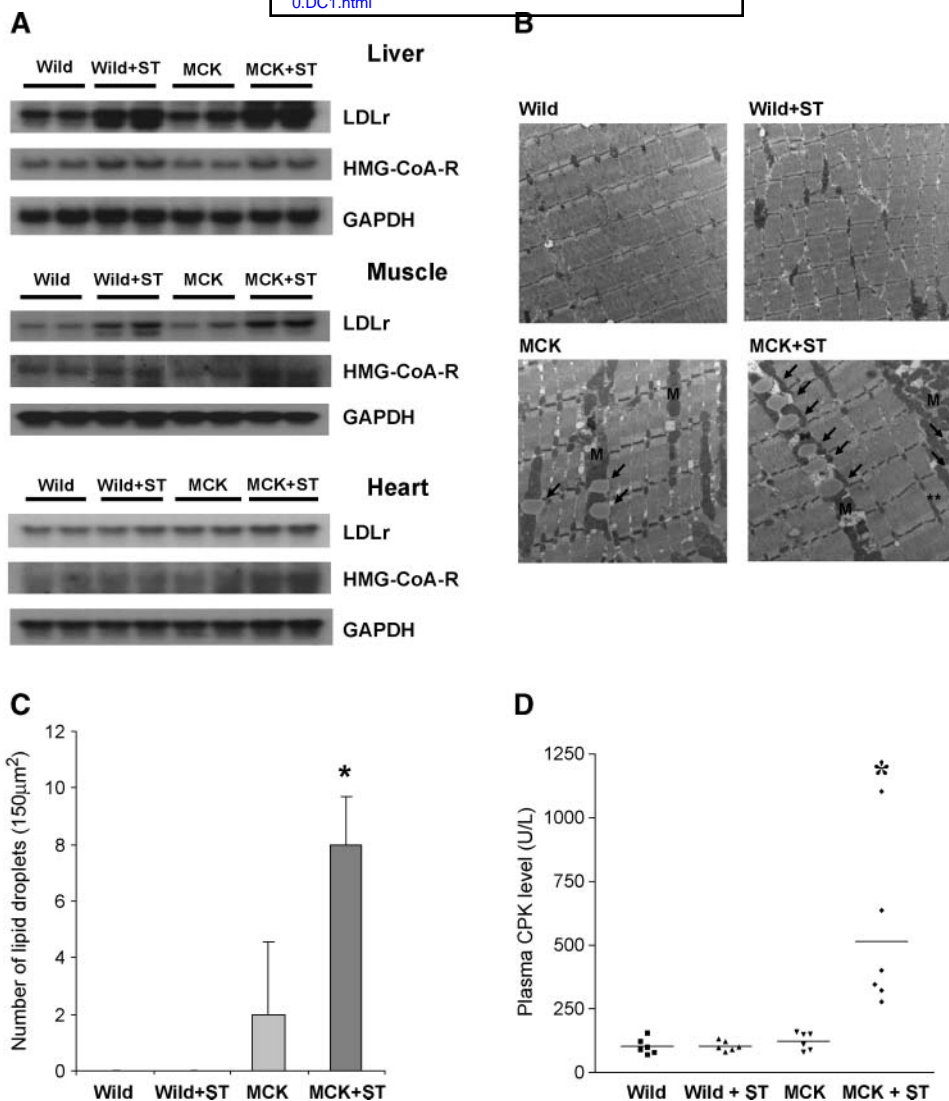


Fig. 4. Effects of statin treatment on muscle gene expression and lipid content. **A:** Gene changes with statin treatment in MCK-LPL mice. Ten micrograms of total RNA was isolated from liver, muscle, and heart, and Northern blot analyses were performed using radiolabeled cDNA probes for *Ldlr* and hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA-R) in wild-type mice (Wild), wild-type mice treated with statin (Wild+ST), MCK-LPL mice (MCK), and MCK-LPL mice treated with statin (MCK+ST). GAPDH is shown as a control for loading. **B:** Electron microscopy of skeletal muscle. Arrows show lipid droplets. M indicates mitochondria. **C:** Number of lipid droplets within 150 μm² area (five for each sample). Values are expressed as means ± SD. * *P* < 0.05 versus MCK-LPL mice. **D:** Plasma creatine phosphokinase (CPK) level in four groups measured with enzymatic tests. * *P* < 0.05 versus MCK-LPL mice.

from chylomicron remnants via a pathway that is exclusive of LPL (20). Whether this process requires scavenger receptors, other members of the LDL receptor superfamily, or some novel process has not been determined.

As we had observed for the heart, LDL receptor expression did not alter muscle LDL uptake. This observation is consistent with other studies showing that LDL receptor-mediated LDL uptake is lower for muscle than for any other tissue of the body (3). LPL overexpression in muscle did not lead to a significant increase in LDL protein uptake. However, skeletal muscle-selective uptake of LDL cholesterol was increased in MCK-LPL mice (6).

We should note that in our studies, the forms of LPL transgenes expressed in the heart and skeletal muscle differed: LPL^{GPI} was anchored to the myocyte surface, whereas nonmutated human LPL was expressed by skeletal muscle. In addition, the relative amounts of expression differed between these transgenes: MCK-LPL leads to an ~8-fold increase in muscle heparin-releasable activity (10), whereas the LPL^{GPI} transgene increases cardiac LPL activity by ~3-fold (9). We suspect that the parenchymal cell-associated form of LPL is most effective for holo-LDL uptake. The mechanisms responsible for LPL-mediated uptake of LDL have been studied in cell culture and may involve LPL serving as a receptor ligand, LPL concentra-

tion of lipoproteins near classical lipoprotein receptors, and/or LPL associating with lipoproteins and mediating internalization along with the turnover of cell surface proteoglycans (21).

One might have expected that the MCK-LPL transgene would downregulate skeletal muscle LDL receptors. However, the uptake of cholesterol via muscle expression of LPL leads to only a small increase in basal cholesterol that may “prime” the muscle to the statin-mediated process but not greatly alter intracellular metabolism. There are several reasons why the small change in muscle cholesterol may be insufficient to downregulate basal LDL receptor expression. 1) The change in cellular cholesterol concentration may be too small. 2) Acquisition of cholesterol via the selective uptake of LDL cholesterol rather than by receptor-mediated endocytosis of holo-LDL might not be equivalent. 3) Skeletal muscle LDL receptors may be maximally downregulated and the residual expression controlled by other factors such as hormones (e.g., insulin and sex hormones), growth factors, and peroxisome-proliferating activator receptor γ (22–25).

Another method to alter LDL receptor expression is via treatment of animals with statins. These drugs inhibit HMG-CoA-R, initially reduce cellular cholesterol, and then increase SREBP transfer to the nucleus, leading to increased expression of genes for cholesterol biosynthesis and LDL receptors (26, 27). As expected, mice treated with simvastatin had increased LDL receptor expression in the liver and greater uptake of plasma LDL. Skeletal muscle in mice treated with simvastatin also had greater expression of LDL receptors and more LDL uptake than did skeletal muscle in untreated mice. Overall, muscle cholesterol content in wild-type mice was unchanged with statin treatment.

MCK-LPL mice with high-level LPL expression develop myopathy even without the addition of statins (10). This had been presumed to occur as a result of excess uptake of fatty acids. The lower level MCK-LPL-expressing mice used in this study did not have obvious muscle pathology. As shown in Fig. 4B, they did have more lipid droplets in skeletal muscle. However, plasma CPK was not increased.

Statin-treated wild-type mice, like most humans, did not experience adverse effects as a result of the muscle upregulation of LDL uptake. However, statin-treated MCK-LPL mice had significantly increased muscle cholesterol concentrations. Electron microscopy of muscles from statin-treated MCK-LPL mice showed increased mitochondria and lipid droplets, and plasma CPK levels were increased. Although this muscle toxicity could have resulted from greater tissue cholesterol levels, increased LDL receptors also mediate the uptake of lipoproteins expressing apolipoprotein E. Thus, in addition to cholesterol, muscles of the statin-treated MCK-LPL mice are likely to have been exposed to greater fluxes of fatty acids and phospholipids, both of which could be toxic.

The effects of simvastatin in our study are similar to the effects of statins in humans. Muscle biopsies from human patients with statin-associated muscle pain show lipid accumulation (28). Moreover, a recent study showed that sub-

jects on high-dose simvastatin actually had increased cholesterol and plant sterols in their muscles (29).

The reasons for statin-induced human muscle toxicity are unknown. Statins may be directly toxic to muscles, if, for example, they block the production of lipid precursors other than LDL. Much of the literature has speculated that statin-induced muscle dysfunction results from a defect in the prenylation (30) or farnesylation (31) of proteins. However, patients on long-term statin treatment have unaltered cholesterol biosynthesis (32, 33), and peripheral cells also compensate for the drug-induced block in synthesis (34). Thus, it is possible that the upregulation of LDL receptors, as illustrated in our animal experiments, is responsible for the abnormal concentrations of muscle lipids in symptomatic statin-treated patients.

Because statin-induced myopathy occurs in a minority of patients, there may be some genetic or physiological predisposition to this side effect. Situations that are associated with the increased expression of LPL, and therefore more muscle cholesterol uptake via this pathway, might also increase the risk of this myopathy. Chronic exercise (35), fasting (36), and the use of fibric acid drugs (37) increase muscle LPL. These conditions might increase side effects/myopathy in muscle because they create a milieu that is similar to that in the MCK-LPL mice. Competitive athletes, who have increased muscle LPL expression (38), are well known to have difficulty with statin therapy (39).

Although statins cause skeletal muscle side effects, there is no evidence that these drugs alter cardiac function, and in our studies statin treatment did not alter heart LDL receptor expression in mice. This is not attributable to a defect in statin uptake into cardiac tissue (40). Moreover, in a preliminary experiment with a small group of mice, statin treatment did not exacerbate the heart dysfunction found in the LPL^{GPI} mice and may have led to some benefit. Ongoing experiments will study this in more detail.

In summary, our data demonstrate that cardiomyocyte-anchored LPL increases LDL uptake. Thus, the site of LPL might markedly affect LPL's function; endothelial cell LPL primarily hydrolyzes circulating TG, whereas parenchymal cell surface LPL mediates holo-lipoprotein uptake. Neither cardiac nor skeletal muscle uptake of LDL is, to a major extent, via the LDL receptor pathway in chow-fed mice. These two muscles differ in their response to statin therapy and the loss of LPL. Only skeletal muscle LDL receptors were upregulated by this drug and by LPL deficiency. In mice with greater LDL uptake resulting from muscle overexpression of LPL, statin therapy increased LDL uptake and tissue cholesterol content and caused muscle damage. Increased cellular cholesterol can lead to dysfunction or even apoptosis (8). It is possible that a similar process is associated with statin muscle dysfunction in humans. However, cardiac muscle dysfunction is not a known side effect of this class of drugs. This, we hypothesize, is attributable to differences in cholesterol delivery pathways used by these two forms of muscle. It should be noted that the levels of LPL increase in our mouse model might not replicate conditions that occur in the unusual patient who develops myopathy. However, because of the

rarity of statin-induced myositis and the likelihood that the disease, which destroys muscle tissue, would alter LPL activity, there is no obvious method to test whether patients with greater LPL activity are more susceptible to this side effect. Thus, our experimental data develop a hypothesis rather than a model for statin-induced myositis. **■**

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