Acute lipoprotein lipase deletion in adult mice leads to dyslipidemia and cardiac dysfunction

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Submitted 9 March 2006; accepted in final form 27 April 2006

The most energetic organ in energy utilization is the cardiac muscle. More than four decades ago, studies of human hearts showed that the primary source of heart fatty acids was from esterified lipids and not free fatty acids (6). Hearts, like all other organs and tissues, can acquire circulating fatty acids from three different sources: albumin-associated free fatty acids, lipolysis-generated fatty acids from lipoprotein triglyceride, and uptake of whole lipoproteins containing triglyceride. We (2) eliminated one of these pathways by producing a 2-wk tamoxifen treatment of MerCreMer (MCM)/Lplflox/flox mice. LPL gene deletion was confirmed by PCR analysis, and LPL mRNA expression was reduced by $\sim$70%. One week after tamoxifen was completed, triglyceride was increased with LPL deletion, 162 ± 53 vs. 91 ± 21 mg/dl, $P < 0.01$. Tamoxifen treatment of Lpflflox/flox mice did not cause a significant increase in triglyceride levels. Four weeks after tamoxifen, MCM/Lplflox/flox mice had triglyceride levels of 190 ± 27 mg/dl, similar to those of mice with prenatal LPL deletion. One week after the tamoxifen, MCM/Lplflox/flox, but not Lpflflox/flox, mice had decreases in carnitine palmitoyl transferase I mRNA (18%) and pyruvate dehydrogenase kinase 4 mRNA (38%). These changes in gene expression became more robust with time. Acute loss of LPL decreased ejection fraction and increased mRNA levels for atrial natriuretic factor. Our studies show that acute loss of LPL can be produced and leads to rapid alteration in gene expression and cardiac dysfunction.

triglyceride; tamoxifen; hypertriglyceridemia; peroxisome proliferator-activated receptor; heart failure; cardiac energetics

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The heart is able to use either glucose or fatty acids for energy. It has been postulated that metabolic flexibility to shift from one energy source to another allows cardiac muscle to adjust to changes in oxygen and blood flow during ischemia (20, 27). In contrast, greater reliance on fatty acid oxidation is associated with mechanical dysfunction (9, 10, 15). These data have been widely interpreted to suggest that greater glucose, and less fatty acid, utilization is beneficial for the heart.

We attempted to produce a system where lipoprotein metabolism and cardiac energetics and function could be studied after acute loss of LPL. Several systems have been developed to induce Cre recombination actions; one of the most effective systems has been to use a Cre recombinase that transfers to the cell nucleus when tamoxifen binds to a mutated estrogen receptor (23, 25, 29). The issues that we addressed were 1) whether the effects of LPL deletion could be differentiated from the increased triglyceride levels that occur with tamoxifen treatment, 2) whether acute loss of LPL led to changes in cardiac gene expression of lipid- and glucose-metabolizing enzymes, and 3) whether this altered cardiac mechanics.

MATERIALS AND METHODS

Generation of MerCreMer (MCM)/Lplflox/flox mice. Mice with cardiomyocyte-selective deletion of LPL were generated by crossing mice homozygous for floxed LPL allele (Fig. 1A) with transgenic MCM mice (23). This transgene permits tamoxifen induction of Cre recombinase only in cardiomyocytes. The presence of the MCM transgene and modified LPL alleles were verified by PCR. For the MCM transgene, the following PCR primers were used: 5'-GTC TGA CTA GGT GTC CTT CT-3' and 5'-CGT CCT GGT GCT CCT GCT GAT A G-3'. The primers used for identifying the loxP site were 5'-CCTGCT-GTAGCATGATCTCAA-3' and 5'-TCTAGGCAGAGGCAGCAG-AGA-3'.

All studies were reviewed and approved by the Columbia University Institutional Animal Care and Use Committee.

Tamoxifen treatment. To induce Cre recombination, male MCM/Lplflox/flox and Lpflflox/flox mice (8–9 wk of age) were treated with 4-hydroxytamoxifen (Sigma, St. Louis, MO) by intraperitoneal injection once a day for 2 wk at a dosage of 20 mg·kg⁻¹·day⁻¹.

Glucose, triglyceride, and cholesterol measurements. Plasma samples were obtained from 6-h-fasted mice before, during, and after the tamoxifen treatment. Glucose was measured directly from the tail tip of anesthetized mice with a glucometer. Total cholesterol and triglyceride levels were measured enzymatically using kits from Infinity (Thermo Electron, Waltham, MA).

Quantitative real-time PCR. Total RNA was isolated from hearts using Trizol reagent (Invitrogen, Carlsbad, CA). The mRNA levels were determined by SYBR Green (Applied Biosystems, Foster City, CA) real-time PCR using 10 ng of total RNA. The primer sequences were atrial natriuretic factor (ANF) sense (S) 5'- CTTGTTGTGTTGTTGACAGCTGACAGTACAA-3' and 5'-TCTAGGCAGAGGCAGCAG-AGA-3'.

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Lpl deletion of the proximal promoter and exon 1. (in the second intron) create a 400-bp product, but only in genes with Cre site (shown as a triangle), these primers create a 734-bp product. Primers A and 700-bp product from a wild-type LPL gene. In a gene containing the first loxP primers A (within the proximal promoter) and B (within exon 1) create a presentation of LPL gene in the absence or presence of Cre recombinase. PCR Lpl by measurement of LPL mRNA by real-time PCR; MCM/heavy-chain (MHC)-Cre transgene (2). This was confirmed at least as effective as chronic LPL loss due to the myosin (acute LPL deletion). The activation of the MCM appears to be 1 wk after tamoxifen treatment.

**RESULTS**

Effects of tamoxifen treatment on LPL gene and mRNA. We used the method described by Pappan et al. (22) to demonstrate the presence of a floxed allele and the excision of the proximal LPL promoter and exon 1. Figure 1B, lane 1, shows the 700-bp product derived from the control LPL gene. Lanes 2 and 3 show the 734-bp product containing the two loxP sites in the Lplflox/flox mice (top). Cre activation leads to a 400-bp product that is shown in lanes 2 (chronic LPL deletion) and 4 (acute LPL deletion). The activation of the MCM appears to be at least as effective as chronic LPL loss due to the myosin heavy-chain (MHC)-Cre transgene (2). This was confirmed by measurement of LPL mRNA by real-time PCR; MCM/ Lplflox/flox hearts had an ~70% reduction (Fig. 1C).

Plasma lipids in treated mice. Although induction of Cre with tamoxifen has been studied in other situations (23, 25, 26, 28), effects of tamoxifen treatment on LPL gene and mRNA. We used the method described by Pappan et al. (22) to demonstrate the presence of a floxed allele and the excision of the proximal LPL promoter and exon 1. Figure 1B, lane 1, shows the 700-bp product derived from the control LPL gene. Lanes 2 and 3 show the 734-bp product containing the two loxP sites in the Lplflox/flox mice (top). Cre activation leads to a 400-bp product that is shown in lanes 2 (chronic LPL deletion) and 4 (acute LPL deletion). The activation of the MCM appears to be at least as effective as chronic LPL loss due to the myosin heavy-chain (MHC)-Cre transgene (2). This was confirmed by measurement of LPL mRNA by real-time PCR; MCM/ Lplflox/flox hearts had an ~70% reduction (Fig. 1C).

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Plasma lipids in treated mice. Although induction of Cre with tamoxifen has been studied in other situations (23, 25, 26, 28), the absence or presence of Cre recombinase. PCR primers A (within the proximal promoter) and B (within exon 1) create a 700-bp product from a wild-type LPL gene. In a gene containing the first loxP site (shown as a triangle), these primers create a 734-bp product. Primers A and C (in the second intron) create a 400-bp product. In a gene containing the first loxP primers A (within the proximal promoter) and B (within exon 1) create a 700-bp product from a wild-type LPL gene. In a gene containing the first loxP site (shown as a triangle), these primers create a 734-bp product. Primers A and C (in the second intron) create a 400-bp product. but only in genes with Cre deletion of the proximal promoter and exon 1. B: PCR screening of DNA from tamoxifen induced MCM/Lplflox/flox hearts showing the PCR products in mice with or without Cre activation either by chronic expression via the myosin heavy-chain promoter (MHC-Cre) or after tamoxifen treatment of MCM/ Lplflox/flox mice (tamoxifen). Lane 1 shows products from hearts of wild-type mice, lane 2 from mice with chronic LPL deletion, lane 3 from MCM/ Lplflox/flox mice not receiving tamoxifen, and lane 4 from MCM/Lplflox/flox mice 1 wk after tamoxifen treatment. C: LPL mRNA assessed by real-time PCR in hearts of tamoxifen-treated Lplflox/flox and MCM/Lplflox/flox mice. Data shown are means ± SD; n = 6 in each group. **P < 0.01 relative to Lplflox/flox.
with regard to lipoprotein metabolism, this is complicated because estrogen-like compounds increase triglyceride levels (13, 16, 31). As shown in Fig. 2, tamoxifen treatment in Lpl\textsuperscript{flox/flox} mice not expressing Cre led to a 27 mg/dl increase in average triglyceride during the tamoxifen treatment, but this change was not significant (\(P < 0.9\)). Average triglyceride returned to control levels by 1 wk after tamoxifen treatment (Fig. 2A). Cholesterol levels were not significantly altered by tamoxifen (Fig. 2B).

In contrast, tamoxifen treatment of MCM/Lpl\textsuperscript{flox/flox} led to greater increased triglycerides of 155 ± 53, 162 ± 49, and 190 ± 27 mg/dl during, 1 wk after, and 4 wk after tamoxifen, respectively. During tamoxifen treatment, triglyceride levels in MCM/Lpl\textsuperscript{flox/flox} mice were significantly increased compared with control, but not tamoxifen-treated, Lpl\textsuperscript{flox/flox} mice. By 1 wk after treatment, triglyceride levels were significantly increased compared with both groups of controls (\(P < 0.05\)). Four weeks after tamoxifen treatment, triglyceride elevations were similar to those found with chronic cardiac-specific LPL knockout (2).

**Gene expression with acute LPL deletion.** To determine whether acute loss of LPL altered cardiac gene expression, we assessed genes involved in lipid and glucose metabolism in hearts from MCM/Lpl\textsuperscript{flox/flox} and Lpl\textsuperscript{flox/flox} 1 wk after completing tamoxifen treatment. In the hearts of tamoxifen-treated MCM/Lpl\textsuperscript{flox/flox} mice, the expression of CPT I was reduced by 18\%, FAS was reduced by 21\%, and CD36 was reduced by 23\% (Fig. 3A). PPAR\(\gamma\) expression decreased; ANF mRNA, a marker of cardiac failure, increased by 27\%. Neither PPAR\(\alpha\), GLUT4, nor GLUT1 were altered (not shown).

In another group of mice, cardiac function and lipid metabolic gene expression were assessed 7 wk after tamoxifen was completed. A similar gene pattern was found, but the reductions in fatty acid oxidation genes were greater, CPT I was reduced by 40\%, CD36 was reduced by 72\%, and PDK-4 was reduced by 72\%. We also compared gene expression between MCM/Lpl\textsuperscript{flox/flox} and chronic, heart-specific, LPL-deleted mice (hLPL0) of the same age. There were no significant differences in CPT I, CD36, PDK-4, and ANF gene expression between two groups (Fig. 3B).

**Heart function in MCM/Lpl\textsuperscript{flox/flox} mice.** Cardiac function was assessed by serial ultrasonography. Although at 1 wk after the end of tamoxifen treatment cardiac function was not altered, 4 wk after tamoxifen treatment the same mice showed reduced fractional shortening. hLPL0 mice matched for age had an almost identical fractional shortening (Fig. 4A). Seven weeks after treatment, areas of cardiac fibrosis were found in MCM/Lpl\textsuperscript{flox/flox} hearts (Fig. 4B). Hearts from wild-type and tamoxifen-treated Lpl\textsuperscript{flox/flox} mice were indistinguishable.

**DISCUSSION**

We studied how acute loss of cardiac LPL affects gene expression and heart function. The objectives of this study were the following. 1) Assess whether the inducible system...
would be effective in LPL gene deletion. At least for LPL in the heart, use of MCM led to an effective deletion of the loxP flanked region, sustained hypertriglyceridemia, and reduced LPL mRNA. 2) Determine whether the effects of tamoxifen would obscure the metabolic effects of loss of cardiac LPL. Tamoxifen by itself did not lead to a significant increase in triglyceride, but its effect was sufficient to conceal the isolated cardiac LPL deletion. 3) Determine whether gene expression and heart dysfunction with cardiac LPL deletion were due to loss of this enzyme during embryogenesis or during the prenatal period. Loss of LPL in 4-mo-old mice (7 wk after treatment) led to changes in cardiac gene expression and function that were similar, but not greater, than those found with chronic LPL deficiency. Loss of cardiac LPL for 1 mo altered heart function; however, the reduction in ejection fraction was equivalent to that in mice of the same age with prenatal loss of LPL.

Estrogens increase plasma triglyceride levels in animals and humans (8, 16, 21). Tamoxifen, a selective estrogen, is a well-established treatment used especially for therapy and prevention of breast cancer. This drug increases triglyceride levels in humans (17, 19, 24). Because tamoxifen induction of Cre leading to acute gene expression is becoming a popular method, it was important to differentiate the effects of this drug from those due to genetic alteration in lipid metabolism. Specifically, we needed to define the degree and chronology of

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**Fig. 3.** Acute loss of LPL in the heart of male mice alters the expression of lipid metabolic genes. A: RNA was isolated from MCM/Lpfllox/flox and Lpfllox/flox hearts 1 wk after tamoxifen treatment was completed. B: gene expression in MCM/Lpfllox/flox, Lpfllox/flox wild-type (WT), and heart-specific LPL-deleted (hLPL0) hearts 7 wk after the conclusion of tamoxifen treatment. Gene expression was assessed by real-time PCR and normalized to β-actin. Data shown are means ± SD; n = 5–7 in each group. *P < 0.05 relative to Lpfllox/flox, **P < 0.05 relative to Lpfllox/flox, ***P < 0.05 relative to WT. PDK-4, pyruvate dehydrogenase-4; CD36, fatty acid transporter; mCPT I, muscle carnitine palmitoyltransferase; FAS, fatty acid synthase; PPARγ, peroxisome proliferator-activated receptor-γ; ANF, atrial natriuretic factor.
the tamoxifen effect in mice. Using the PCR-based system developed by Pappan et al. (22), we found that tamoxifen treatment of MCM/Lplflox/flox mice led to DNA deletion and LPL mRNA loss that was equivalent to that found with chronic LPL deficiency due to MHC-Cre expression (2).

Although tamoxifen increased plasma triglyceride, that effect was modest (a 30% increase), variable, and transient. By 1 wk after treatment, plasma triglyceride had returned to baseline. However, during tamoxifen treatment, we could not distinguish triglyceride differences between mice with cardiac LPL knockout and controls. Only after the tamoxifen was discontinued and triglyceride in control mice returned to baseline was evidence of gene deletion apparent. Thus investigators using this system to study lipid metabolism need to assess changes over time and after discontinuation of tamoxifen.

The expression of the MHC-Cre has been reported previously (1) and is restricted to cardiomyocytes. Because the heart is composed of a number of cell types, including myofibroblasts, endothelial cells, and macrophages, residual LPL expression was expected. It has been estimated that ≈27% of the LPL expressed in the heart is due to macrophages (5). It is also possible that some of the residual LPL was due to partial loss of cardiomyocyte LPL. Nonetheless, the deletion was sufficient to lead to changes in function and gene expression in the heart. This system to induce LPL loss can now be used to study acute loss of fatty acid uptake in mice with established disease due to environmental or other genetic manipulations.

Do hearts adjust differently to acute vs. chronic LPL deficiency? By assessing gene expression and cardiac function 1 wk and 6–7 wk after LPL deletion, we were able to show that LPL loss rapidly reduces expression of genes regulating fatty acid oxidation. This was also found with more chronic LPL loss and might be due to the loss of LPL-generated ligands for PPARs (32). We also noted that more chronic loss of LPL led to greater differences in expression of PPARα-regulated genes. Perhaps this reflects changes in the requirements for cardiac fatty acids during aging (14). In the hLPL0 mice, we reported that glucose uptake was elevated five- to eightfold. Thus LPL-deficient hearts that are unable to acquire lipoprotein fatty acids adjust by switching to an alternative fuel.

As we have found with prenatal LPL loss (3), tamoxifen-treated MCM/Lplflox/flox hearts developed cardiac dysfunction and increased fibrosis. We had initially hypothesized that acute LPL loss would lead to more profound changes; such a result would suggest that hearts chronically deficient in fatty acid uptake adjust. We were incorrect; both LPL loss and aging appear to be necessary. Drugs that inhibit cardiac fatty acid oxidation have been developed and appear to be useful in short-term studies where generation of cardiac energy might be limited by oxygen availability (4, 12, 18); generation of ATP from glucose requires less oxygen. However, our data suggest that prolonged loss of fatty acid utilization could be harmful.

In summary, we created mice with an acute loss of LPL in the heart. These mice develop similar changes in plasma lipids,
cardiac genes, and heart function to those found with prenatal loss of this enzyme in the heart. This confirms that developed hearts are affected by loss of LPL and shows that loss of this enzyme during development and in the prenatal period is not the primary reason for the metabolic and functional changes seen with chronic LPL loss. Moreover, it confirms the need for fatty acids in the adult heart and suggests that interventions that inhibit the heart’s ability to utilize fatty acids could have deleterious effects (3). Acute loss of LPL occurs with infections (11). In addition, some, but not all, studies (7) have found reduced cardiac LPL activity with starvation and diabetes. Perhaps such changes in LPL actions could, in part, be responsible for acute alterations in cardiac function.

ACKNOWLEDGEMENTS

These studies were supported by Grants HL-73029 and HL-45095 from the National Heart, Lung, and Blood Institute.

REFERENCES