Reduction of plasma triglycerides in apolipoprotein C-II transgenic mice overexpressing lipoprotein lipase in muscle

Leslie K. Pulawa, Dalan R. Jensen, Alison Coates,[1] and Robert H. Eckel[2]

Division of Endocrinology, Metabolism, and Diabetes, University of Colorado at Denver and Health Sciences Center, Aurora, CO

Abstract LPL and its specific physiological activator, apolipoprotein C-II (apoC-II), regulate the hydrolysis of triglycerides (TGs) from circulating TG-rich lipoproteins. Previously, we developed a skeletal muscle-specific LPL transgenic mouse that had lower plasma TG levels. ApoC-II transgenic mice develop hypertriglyceridemia attributed to delayed clearance. To investigate whether overexpression of LPL could correct this apoC-II-induced hypertriglyceridemia, mice with overexpression of human apoC-II (CII) were cross-bred with mice with two levels of muscle-specific human LPL overexpression (LPL-L or LPL-H). Plasma TG levels were 319 ± 39 mg/dl in CII mice and 39 ± 5 mg/dl in wild-type mice. Compared with CII mice, apoC-II transgenic mice with the higher level of LPL overexpression (CIILPL-H) had a 50% reduction in plasma TG levels ($P = 0.013$). Heart LPL activity was reduced by ~30% in mice with the human apoC-II transgene, which accompanied a more modest 10% decrease in total LPL protein. Overexpression of human LPL in skeletal muscle resulted in dose-dependent reduction of plasma TGs in apoC-II transgenic mice. Along with plasma apoC-II concentrations, heart and skeletal muscle LPL activities were predictors of plasma TGs. These data suggest that mice with the human apoC-II transgene may have alterations in the expression/activity of endogenous LPL in the heart. Furthermore, the decrease of LPL activity in the heart, along with the inhibitory effects of excess apoC-II, may contribute to the hypertriglyceridemia observed in apoC-II transgenic mice.—Pulawa, L. K., D. R. Jensen, A. Coates, and R. H. Eckel. **Reduction of plasma triglycerides in apolipoprotein C-II transgenic mice overexpressing lipoprotein lipase in muscle.** ***J. Lipid Res.*** **2007.** 48: **145–151.**

Supplementary key words hypertriglyceridemia • lipid metabolism • heparin-releasable lipoprotein lipase activity • heart • fasting

As the rate-limiting enzyme in the hydrolysis of triglycerides (TGs) from circulating TG-rich lipoproteins, LPL is a key enzyme in lipid metabolism. Found in most tissues, LPL is most abundant in adipose tissue, heart, and skeletal muscle. Anchored to the endothelium by heparan proteoglycans within the capillary beds of these tissues, active lipase hydrolyzes the TG core of lipoproteins into FFAs, monoglycerides, and remnant lipoproteins. The lipoprotein-derived FFAs are then available for uptake and use by extrahepatic tissues for either storage or oxidation. Observations from experimental alterations in LPL activity levels and localization underscore LPL's role as a "gatekeeper" for partitioning lipoprotein-derived FFAs between tissues. Perturbations in normal LPL activity levels and distribution in mice have resulted in changes in body composition and lipid and glucose metabolism (1–5).

Apolipoprotein C-II (apoC-II) is the specific physiological activator necessary for LPL activity; therefore, it plays a central role in the metabolism of plasma TG. Patients with defects in apoC-II present with high circulating levels of TGs comparable to those seen with LPL deficiency (6–9). However, high concentrations of free apoC-II protein have been shown to inhibit LPL activity in vitro (10). Overexpressing human apoC-II in mice resulted in hypertriglyceridemia attributed to the delayed clearance of VLDL TGs, and human apoC-II levels were highly correlated with plasma TGs (11). In humans, one case of drug-resistant hypertriglyceridemia has been reported in a patient with increased levels of apoC-II (12). To date, the mechanisms by which physiological levels of apoC-II activate and excess apoC-II inhibits LPL are not fully understood.

Our laboratory has previously generated a transgenic mouse with muscle-specific LPL overexpression that results in a reduction of plasma TG levels (1, 2). To further investigate the interaction of apoC-II and LPL, apoC-II transgenic mice were cross-bred with muscle-specific LPL transgenic mice.

Abbreviations: apoC-II, apolipoprotein C-II; HSPG, heparan sulfate proteoglycan; TG, triglyceride; WT, wild-type.

[1] Present address of A. Coates: Nutritional Physiology Research Centre, School of Health Sciences, University of South Australia, Adelaide, South Australia, Australia.

[2] To whom correspondence should be addressed.
e-mail: robert.eckel@uchsc.edu

Manuscript received 28 October 2005 and in revised form 28 August 2006.

Published, JLR Papers in Press, October 3, 2006.
DOI 10.1194/jlr.M600384-JLR200

MATERIALS AND METHODS

Animals

Studies were conducted in accordance with protocols approved by the Animal Care and Use Committee at the University of Colorado at Denver and Health Sciences Center. Mice were housed at ~20°C on a 12 h/12 h light/dark photoperiod and provided standard rodent chow and water ad libitum. Previously, transgenic mice with human LPL overexpression in skeletal muscle were generated and characterized (LPL-L) (2). From a separate founder mouse, a second line was generated with higher skeletal muscle overexpression of human LPL (LPL-H). Both LPL-L and LPL-H mice were crossed with transgenic mice expressing human apoC-II (CII) (11). The resultant offspring were apoC-II transgenic (CII), LPL transgenic (LPL-L and LPL-H), double transgenic (CIILPL-L and CIILPL-H), and wild-type (WT) mice. Both male and female mice (3–12 months of age) were included in the studies. In procedures in which mice were anesthetized, mice received an intraperitoneal injection of Avertin (2,2,2-tribromoethanol; 250 mg/kg). In studies in which mice were fasted, food was removed between 8 and 10 AM.

Cell culture

Stably transfected C2C12 myoblasts overexpressing human LPL (13) were plated and expanded to confluence on 12-well plates.

Tissue and plasma LPL activity

Heparin-releasable LPL activity was measured in heart and skeletal muscle (gastrocnemius) as described previously (14). Briefly, tissues from anesthetized mice after a 4 h fast were minced in cold Krebs-Ringer-phosphate buffer (KRP), pH 7.4, to ~3 mm^3 pieces, and bundles of tissue pieces weighing 35–50 mg were incubated in a shaking 37°C water bath for 45 min in 0.4 ml of KRP with 15 μg/ml heparin. A 100 μl aliquot was removed and incubated with 100 μl of a [^{14}C]triolein phosphatidylcholine-stabilized substrate. After another 45 min incubation at 37°C, the reaction was solubilized, and ^{14}C-labeled fatty acids were partitioned according to the method of Belfrage and Vaughn (15). A 500 μl aliquot of the resulting aqueous supernatant was counted by β-scintillation (Beckman; LS6000TA). [^{14}C]oleic acid was used to control for extraction efficiency. LPL activity was expressed as nanomoles of FFA per minute per gram of tissue.

Preheparin and postheparin plasma LPL activities were measured as described previously (16) with minor modifications. Heparin (100 U/kg) was administered to anesthetized mice after a 4 h fast by cardiac injection, and a blood sample was taken by cardiac puncture 5 min after injection. The preheparin LPL activity assay was performed using fresh plasma at a 1:2 dilution, whereas plasma for the postheparin LPL activity assay was stored at −20°C until analysis using a 1:50 dilution. Plasma samples for the total lipase assay were diluted using KRP, and samples for the hepatic TG lipase assay were diluted in KRP with NaCl for a final concentration of 1 M NaCl. LPL activity was expressed as nanomoles of FFA per hour per milliliter of plasma.

Cell LPL activity

Cell surface LPL activity was measured in LPL-overexpressing C2C12 cells using [^{14}C]triolein phosphatidylcholine-stabilized substrates with four different concentrations of apoC-II protein. Substrates were prepared and the assay performed as described previously (14) with modifications. Purified human apoC-II (Athens Research and Technology, Athens, GA) dissolved in saline was used as the apoC-II source for substrates in place of human sera. The final concentrations of apoC-II in the four substrates were 2.25 μg/ml (1×), 22.5 μg/ml (10×), 45 μg/ml (20×), and 150 μg/ml (67×). After reaching confluence, cells were washed twice with PBS. Four hundred microliters of the 1×, 10×, 20×, or 67× substrate was applied directly to the cells. After the cells were incubated with the substrate at 37°C and 5% CO_2 for 45 min, a 100 μl aliquot was removed, combined with 100 μl of KRP, and solubilized. A 500 μl aliquot of the resulting aqueous supernatant was counted by β-scintillation (Beckman; LS6000TA). [^{14}C]oleic acid was used to control for extraction efficiency. LPL activity was normalized to total protein as measured by the bicinchoninic acid method (Pierce, Rockford, IL).

Total LPL protein

Total LPL protein was measured in heart, skeletal muscle (gastrocnemius), and white adipose tissue after a 4 h fast. Tissues were homogenized in ice-cold RIPA buffer [50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, and 1:1,000 Protease Inhibitor Cocktail (P8340; Sigma, St. Louis, MO)]. The homogenate was incubated on ice for 30 min and then centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was filtered through two layers of cheesecloth. Protein concentration was determined by the bicinchoninic acid method (Pierce). Twenty micrograms of protein was electrophoresed on a 4–15% Tris-HCl gradient gel and transferred onto polyvinylidene difluoride membranes. The membranes were blocked in 5% nonfat milk in TBS-T (20 mM Tris, 500 mM NaCl, 0.05% Tween-20, pH 7.6) for 1 h at room temperature. For detection of LPL protein, blots were incubated overnight at 4°C with purified IgG obtained from a goat anti-rat LPL antiserum (17) at a dilution of 1:100 in 2% nonfat milk in TBS-T (pH 7.6), washed, and then incubated for 1 h with a 1:5,000 dilution of secondary antibody (donkey anti-goat IgG-HRP SC-2020; Santa Cruz Biotechnology, Santa Cruz, CA). The antibody was detected by chemiluminescence (ECL; Amersham Biosciences, Piscataway, NJ) and visualized on film. Western blots were analyzed by AlphaEaseFC (Alpha Innotech Corp., San Leandro, CA).

Real-time RT-PCR

Murine LPL mRNA levels in heart were determined by real-time RT-PCR in mice after a 4 h fast. Total RNA was isolated from tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). The cDNA was produced from 1 μg of total RNA by reverse transcription using SuperScript II (Invitrogen) incubated at 42°C for 60 min, and the reaction was stopped with a 5 min incubation at 99°C. An ABI Prism 7700 sequence detector (Perkin-Elmer Corp./Applied Biosystems, Foster City, CA) was used for continuous measurement of the fluorescence spectra during PCR amplification. Primers and probe for murine LPL were designed with the assistance of the Prism 7700 sequence detection software (Primer Express; Perkin-Elmer Corp./Applied Biosystems). The following primer/probe sequences were used: LPL forward primer, 5′-TTC CAG CCA GGA TGC AAC A-3′; LPL reverse primer, 5′-GGT CCA CGT CTC CGA GTC C-3′; and probe, 5′-AGA AGC CAT CCG TGT GAT TGC AGA GAG-3′. The TaqMan probe (Perkin-Elmer) had the 5′ end labeled with 6-carboxyfluorescein and the 3′ end labeled with 6-caboxy-tetramethylrhodamine. Subsequently, 40 cycles of amplification were performed at 95°C for 15 s and 60°C for 1 min. Quantities of murine LPL in test samples were normalized to the corresponding 18S rRNA (Perkin-Elmer Corp./Applied Biosystems).

Body composition and plasma measurements

Mice were anesthetized after a 4 h fast, and body composition was measured by dual-energy X-ray absorptiometry using the PIXImus2 mouse densitometer (Lunar Corp., Madison, WI).

Blood was collected by cardiac puncture and spun at 4°C, and the resulting plasma was stored at −20°C until further analysis. Glucose was measured using the Analox GM7 (Analox Instruments USA, Lunenburg, MA). TGs were measured using an enzymatic, colorimetric assay (Sigma). Human apoC-II was measured by Linco Diagnostic Services (St. Charles, MO) using the Human Apolipoprotein LINCOplex kit (Linco Research). Two separate Human Apolipoprotein LINCOplex kits were used to evaluate apoC-II levels in mice from the CII×LPL-L cross and the CII×LPL-H cross.

Changes in plasma TG were followed in mice over an 8 h fast. Blood was collected from the tail of awake mice before the fast and at 2, 4, 6, and 8 h of fasting. Plasma was stored at −20°C until further analysis.

Statistical analyses

Results are presented as means ± SEM. Statistical analyses were performed using SigmaStat 2.03 (San Rafael, CA). Plasma TG data were not normally distributed and were log-transformed for statistical analyses. Sets of data were analyzed using *t*-test, one-way ANOVA (with Tukey pairwise comparisons), two-way repeated-measures ANOVA (with Tukey pairwise comparisons), or stepwise forward regression. $P < 0.05$ was considered statistically significant.

RESULTS

Body composition and glucose

Body composition was assessed by dual-energy X-ray absorptiometry to determine whether overexpression of apoC-II altered whole body fat deposition. Body weight, lean mass, fat mass, and percentage fat mass were not different between all four groups in either the CII×LPL-L cross or the CII×LPL-H cross (**Table 1**). Plasma glucose levels also were not different between mice from the CII×LPL-L cross (WT, 11.3 ± 1.4 mmol; CII, 13.4 ± 1.0 mmol; LPL-L, 13.3 ± 1.6 mmol; CIILPL-L, 14.0 ± 0.9 mmol) or the CII×LPL-H cross (WT, 14.1 ± 0.8 mmol; CII, 13.2 ± 0.4 mmol; LPL-H, 13.8 ± 0.8 mmol; CIILPL-H, 13.3 ± 0.8 mmol).

Tissue and plasma LPL activity

The two mouse models of skeletal muscle-specific LPL overexpression (LPL-L and LPL-H) resulted in two different levels of LPL activity in skeletal muscle (gastrocnemius) (**Fig. 1**). Skeletal muscle LPL activity levels were ~5- to 8-fold higher in LPL-L and CIILPL-L mice compared with WT and CII mice. LPL activity measured in the skeletal muscle of LPL-H and CIILPL-H mice was ~13- to 17-fold higher than in WT and CII mice. LPL-H mice had approximately double the LPL activity in their skeletal muscle compared with LPL-L mice.

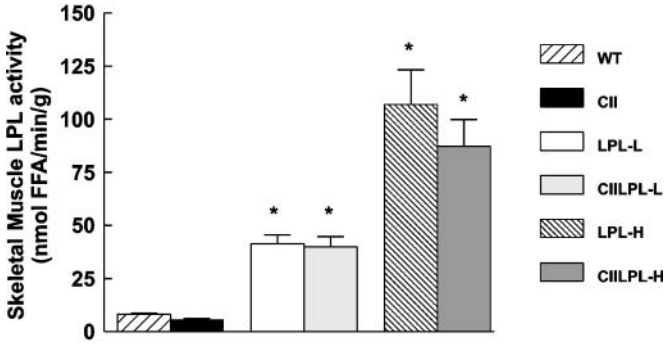

Fig. 1. Skeletal muscle LPL activity in wild-type (WT; n = 17), CII (n = 24), LPL-L (n = 11), CIILPL-L (n = 12), LPL-H (n = 12), and CIILPL-H (n = 12) mice. See Methods for descriptions of mouse lines. LPL was overexpressed at two different levels in mouse skeletal muscle (gastrocnemius). Both levels of overexpression resulted in significantly higher LPL activity in the LPL transgenic mice (LPL-L and LPL-H) and the double transgenic mice (CIILPL-L and CIILPL-H) compared with WT animals. Values shown are means ± SEM. * $P < 0.05$ versus WT.

LPL overexpression was limited to the skeletal muscle and did not result in increased LPL activity in heart. There was no significant difference between heart LPL activity in LPL-H compared with WT mice (**Fig. 2**). Interestingly, heart LPL activity was decreased significantly in mice containing the apoC-II transgene (CII and CIILPL-H) compared with WT and LPL mice (Fig. 2). Heart LPL activity was decreased by 35% in CII compared with WT mice ($P < 0.001$) and was decreased by 30% in CIILPL-H compared with LPL-H mice ($P = 0.015$). Comparisons between CII, WT, CIILPL-L, and LPL-L mice showed a similar trend, but the differences were not significant (data not shown).

As a reflection of functional LPL at endothelial surfaces of the whole body, LPL activity in postheparin plasma was measured in CII and WT mice. Plasma samples were diluted 1:50 to keep the readings within the range of the assay and to eliminate apoC-II inhibition of LPL activity in the CII mouse plasma samples. Although postheparin plasma LPL activity was 55% lower in CII mice (2,122 ± 157 nmol FFA/h/ml; n = 3) compared with WT mice (4,791 ± 1,366 nmol FFA/h/ml; n = 4), the difference was not significant ($P = 0.16$). LPL activity was not detectable in the preheparin plasma of CII, LPL-H, and WT mice.

TABLE 1. Body composition

	CII×LPL-L				CII×LPL-H			
Variable	WT (n = 10)	CII (n = 11)	LPL-L (n = 11)	CIILPL-L (n = 12)	WT (n = 7)	CII (n = 13)	LPL-H (n = 12)	CIILPL-H (n = 12)
Body weight (g)	25.4 ± 1.2	27.4 ± 2.0	26.8 ± 1.1	27.4 ± 1.7	26.1 ± 1.9	23.5 ± 0.9	26.3 ± 1.6	23.8 ± 0.9
Lean mass (g)	20.8 ± 0.9	21.8 ± 1.9	21.8 ± 0.8	22.2 ± 1.3	20.4 ± 1.4	18.6 ± 0.7	20.5 ± 1.0	19.0 ± 0.8
Fat mass (g)	3.9 ± 0.4	4.7 ± 1.0	4.3 ± 0.4	4.5 ± 0.6	4.5 ± 0.7	3.6 ± 0.3	4.6 ± 0.7	3.5 ± 0.2
Percentage fat mass	15.5 ± 1.0	16.9 ± 1.6	16.1 ± 0.9	16.7 ± 1.2	17.4 ± 1.5	15.9 ± 0.7	17.5 ± 1.3	15.5 ± 0.5

See Methods for descriptions of mouse lines.

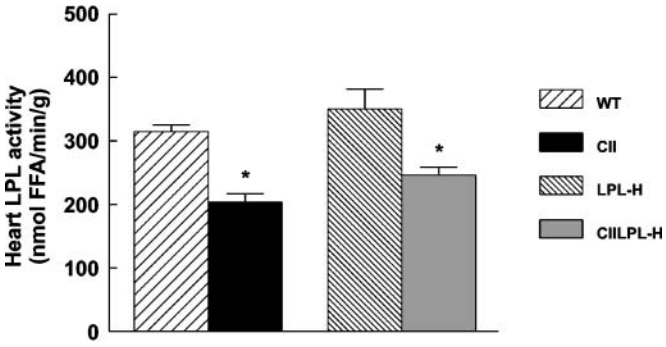

Fig. 2. Heart LPL activity in WT (n = 7), CII (n = 13), LPL-H (n = 12), and CIILPL-H (n = 12) mice. Mice with the apolipoprotein C-II (apoC-II) transgene (CII and CIILPL-H) showed a significant 30–35% decrease in heart muscle LPL activity compared with WT littermates. The presence of the LPL transgene alone (LPL-H) did not alter LPL activity levels in the heart. Values shown are means ± SEM. * $P < 0.05$ versus WT.

The total lipase activity measured in these samples was largely the result of hepatic TG lipase (data not shown).

Murine LPL expression

To determine whether the reduction in LPL activity in heart of mice carrying the human apoC-II transgene was the result of decreased endogenous LPL expression, murine LPL mRNA levels were determined by real-time RT-PCR in mice from the CII×LPL-H cross. The ratio of murine LPL RNA to 18S RNA was 1,107 ± 149 fg/ng in WT (n = 4), 1,652 ± 117 fg/ng in CII (n = 4), 1,347 ± 151 fg/ng in LPL-H (n = 7), and 1,840 ± 211 fg/ng in CIILPL-H (n = 6) mice. Contrary to the LPL activity results, mouse LPL mRNA was upregulated in heart of CII and CIILPL-H mice compared with WT and LPL mice (ANOVA; $P = 0.047$), and when pairwise comparisons were performed, the increase in LPL mRNA of CIILPL-H compared with WT mice was just above the level set for significance (Tukey; $P = 0.051$).

Although endogenous LPL mRNA did not decrease in the heart of CII mice, there was a decrease in total LPL protein in heart and skeletal muscle. CII mice had 10% and 20% less LPL protein in the heart ($P = 0.004$) (**Fig. 3A**) and skeletal muscle ($P = 0.024$) (Fig. 3B), respectively, compared with WT mice. There was no significant difference in total LPL protein levels in white adipose tissue (Fig. 3C).

Human apoC-II and plasma TGs

Human apoC-II levels in mice transgenic for human apoC-II (CII, CIILPL-L, and CIILPL-H) were variable and ranged from 18.5 to 198.7 mg/dl. From the CII×LPL-L cross, the mean human apoC-II levels were 70.9 ± 7.3 mg/dl in CII and 67.2 ± 12.7 mg/dl in CIILPL-L mice. From the CII×LPL-H cross, the mean human apoC-II levels were 109.2 ± 7.1 mg/dl in CII and 93.9 ± 14.0 mg/dl in CIILPL-H mice.

Plasma TG levels in CII mice from both the CII×LPL-L and CII×LPL-H crosses were ~8-fold higher compared

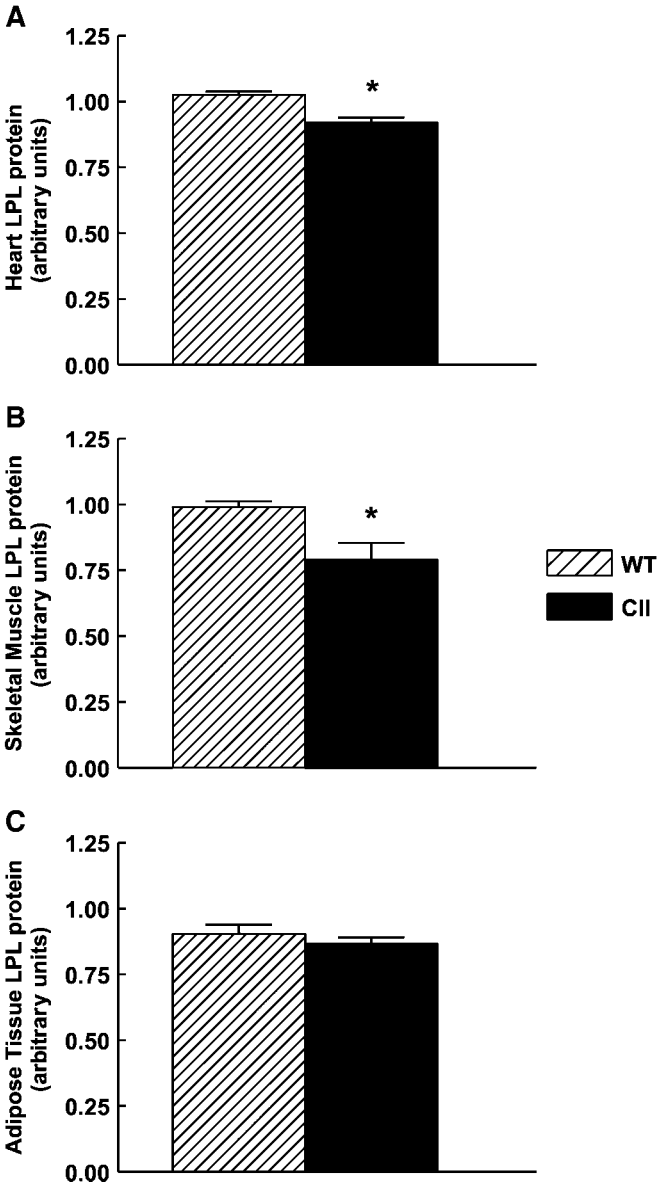

Fig. 3. Total LPL in WT (n = 4) and CII (n = 4) mice. Total LPL protein was measured by Western blot in heart (A), skeletal muscle (gastrocnemius) (B), and white adipose tissue (C). LPL was significantly lower in heart and skeletal muscle of CII compared with WT mice, but there was no difference in adipose tissue. Values shown are means ± SEM. * $P < 0.05$ versus WT.

with those of their respective WT littermates (**Fig. 4A**) ($P < 0.001$ for both comparisons). Compared with CII, CIILPL-L plasma TG levels were 30% lower, although not significantly different. However, with higher levels of LPL overexpression in CIILPL-H mice, plasma TGs were significantly reduced by nearly 50% compared with CII mice (Fig. 4A) ($P = 0.013$). Human apoC-II levels were strongly correlated with log-transformed plasma TG in CII, CIILPL-L, and CIILPL-H mice (Fig. 4B) ($r = 0.681$, $P < 0.0001$).

To determine whether tissue LPL activities along with human apoC-II levels were predictive of plasma TG concentrations, a forward stepwise regression was performed using data only from mice with the human apoC-II transgene (CII, CIILPL-L, and CIILPL-H mice). The depen-

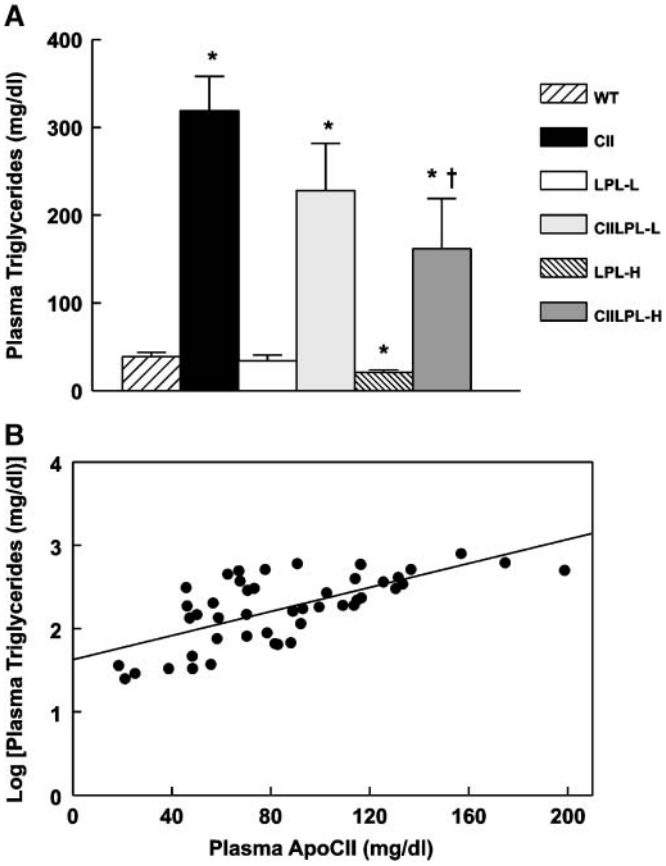

Fig. 4. A: Plasma triglycerides (TGs) in WT (n = 17), CII (n = 24), LPL-L (n = 11), CIILPL-L (n = 12), LPL-H (n = 12), and CIILPL-H (n = 12) mice. The human apoC-II transgene resulted in an ~8-fold increase of plasma TGs in transgenic mice (CII). Addition of the human LPL transgene in skeletal muscle to apoC-II transgenic mice resulted in a dose-dependent decrease in plasma TGs of CIILPL-L and CIILPL-H mice. The plasma TG levels of CIILPL-H mice were half those of CII mice. Values shown are means ± SEM. * $P < 0.05$ versus WT; † $P < 0.05$ versus CII. B: Log-transformed plasma TG concentrations were strongly correlated to plasma human apoC-II levels in CII (n = 24), CIILPL-L (n = 12), and CIILPL-H (n = 12) mice ($r = 0.681$, $P < 0.0001$).

dent variable was log-transformed plasma TG [log (TG)], and the independent variables were human apoC-II levels (apoC-II), heart LPL activity (hLPL), and skeletal muscle LPL activity (smLPL). Using this model, human apoC-II levels, skeletal muscle LPL activity, and heart LPL activity were predictors of plasma TG: log(TG) = 2.128 + 0.00726(apoC-II) − 0.00226(hLPL) − 0.00262(smLPL) ($R^2 = 0.725$).

Mice from the CII×LPL-H cross were used to follow the changes in plasma TGs during an 8 h fast. During the course of the fast, plasma TGs decreased in all groups (**Fig. 5**). Addition of the LPL transgene to the apoC-II transgene in CIILPL-H mice resulted in significantly lower plasma TGs compared with CII mice over the entire fasting period ($P = 0.001$). CIILPL-H plasma TGs were not significantly different from those of WT mice during the 8 h fast. LPL-H plasma TGs were significantly lower compared with WT levels ($P = 0.045$).

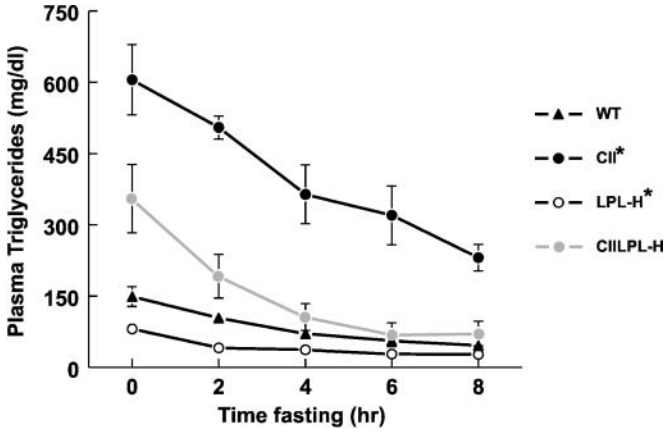

Fig. 5. Plasma TGs during an 8 h fast in WT (n = 4), CII (n = 4), LPL-H (n = 7), and CIILPL-H (n = 5) mice. Over the entire fast, plasma TGs were significantly higher in CII compared with WT mice. Plasma TGs were not different between CIILPL-H and WT mice. Values shown are means ± SEM. * $P < 0.05$ versus WT.

Effects of apoC-II in vitro

To further study the impact of increasing amounts of apoC-II in a setting of LPL overexpression, we used a stably transfected C2C12 cell line that overexpresses active LPL at the cell surface. Purified apoC-II protein was added to create [^{14}C]triolein phosphatidylcholine-stabilized substrates with four different concentrations of apoC-II. The 1× substrate (2.25 μg/ml apoC-II) corresponded to a substrate in which normal human serum was the apoC-II source (normal human range, 1.0–5.5 mg/dl) and was regarded as the control substrate. The remaining three substrates received increasing amounts of the protein to correspond to apoC-II sources from CII mice with the lowest, middle, and highest levels of apoC-II measured in the plasma. LPL activity increased 27% and 28% when the assay was performed with the 10× and 20× substrates compared with the 1× substrate ($P < 0.001$ for both) (**Fig. 6**). However, LPL activity decreased 96% when the substrate with the highest level of apoC-II (>60-fold higher than the 1× substrate) was applied to the cells ($P < 0.001$).

DISCUSSION

ApoC-II is the potent physiological activator of LPL, and together, they are the primary factors in regulating lipoprotein-derived TG hydrolysis. ApoC-II has also been shown to act as an inhibitor of LPL at higher concentrations (10), although the mechanisms by which this activation and inhibition take place remain poorly understood. Here, we report on two different levels of skeletal muscle-specific LPL overexpression (LPL-L and LPL-H) and their impact on apoC-II transgene-induced hypertriglyceridemia. The hypertriglyceridemia reported in apoC-II transgenic mice was attributed to reduced lipoprotein clearance caused by defects in VLDL hydrolysis and not overproduction (11). Our laboratory demonstrated that the overexpression of LPL in skeletal muscle was able to partially overcome the hydrolysis defect with the overex-

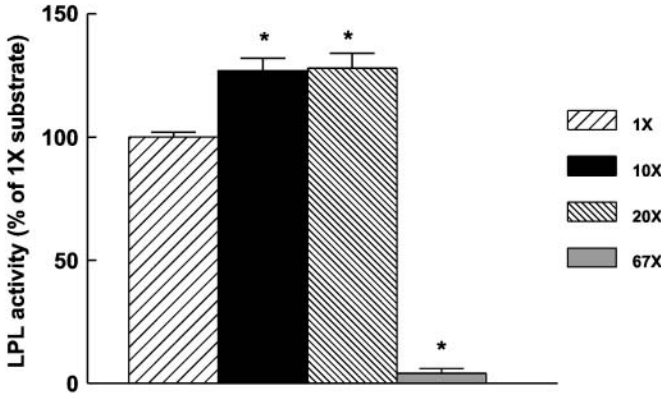

Fig. 6. Effect of apoC-II on LPL activity in C2C12 LPL-overexpressing myoblasts. Increasing levels of purified human apoC-II were added to [^{14}C]triolein phosphatidylcholine-stabilized substrates to create substrates with final concentrations of 2.25 μg/ml (1×; n = 7), 22.5 μg/ml (10×; n = 4), 45 μg/ml (20×; n = 4), and 150 μg/ml (67×; n = 3) apoC-II. LPL activity increased using the 10× and 20× substrates but decreased by 96% with the highest level of apoC-II (67×). Values shown are means ± SEM. * $P < 0.05$ versus 1× substrate.

pression of LPL in skeletal muscle. This improvement was achieved in a dose-dependent manner, as a greater decrease in plasma TG was attained with the higher level of LPL overexpression.

As reported previously by Shachter et al. (11), not only did apoC-II transgenic mice show marked increases in chylomicrons and VLDL TGs, but the apolipoprotein composition of these lipoproteins was altered, with striking increases in the apoCs and a concomitant decrease in apoE. It has been suggested that the lack of apoE resulted in weaker lipoprotein binding to cell surface LPL via heparan sulfate proteoglycans (HSPGs). However, the ablation of apoE in mice only results in mildly increased plasma TG levels (18, 19). In the absence of apoE, VLDL is still able to bind to HSPGs with high affinity through lipase-promoted binding (LPL and hepatic TG lipase) (20), and, in fact, increasing amounts of apoE on nascent VLDL particles inhibit the hydrolysis of TGs mediated by the HSPG-LPL complex and LPL in solution (21). Although apoE is required for efficient VLDL degradation by lipoprotein receptor-mediated endocytosis, the lack of apoE in chylomicrons/VLDLs cannot account for the level of hypertriglyceridemia observed in apoC-II transgenic mice.

Excess apoC-II may directly inhibit lipoprotein access to LPL and HSPG by steric hindrance or electrostatic interference. VLDL from apoC-II transgenic mice was defective in its interaction with heparin-Sepharose; however, the activity of purified mouse LPL was not altered in the presence of apoC-II transgenic plasma in an ex vivo LPL activity assay (11). Our laboratory observed that LPL activity on the surface of LPL-overexpressing C2C12 myoblasts was greatly inhibited by apoC-II levels similar to the highest concentration found in apoC-II transgenic mouse plasma. One possibility is that excess apoC-II may cause the lipoprotein particle to become less "sticky" (electrostatic interference) to the HSPG and thus to limit LPL access to the TG core of chylomicrons and VLDLs. Reducing the length of time LPL has to act on the lipoprotein may explain the accumulation of larger TG-rich VLDL particles observed in apoC-II transgenic mice. Overexpressing LPL in the skeletal muscle would increase the number of LPL molecules per lipoprotein particle that could act simultaneously for the same duration of limited contact, thus increasing the number of hydrolytic events per time period. During the first 6 h of the 8 h fast, the rate of TG disappearance was the same for both CII and CIILPL-H animals (~50 mg/dl/h), suggesting that the kinetics for LPL were the same during the fast.

The heart is also a major site of TG hydrolysis and expresses LPL in abundance (22, 23). Previous studies have shown that heart-specific LPL ablation in mice results in significant hypertriglyceridemia (24); conversely, when LPL expression is limited solely to the heart, mice have normal plasma TG and HDL cholesterol levels (25). Unexpectedly, we observed a significant reduction of heart LPL activity in the human apoC-II transgenic mouse. Upon examining the expression of the endogenous mouse LPL gene in heart, we found that apoC-II transgenic mice have increased murine LPL mRNA. Much of the regulation of LPL, however, occurs at the posttranslational level. More reflective of the loss in LPL activity was the significant, although modest, 10% decrease in total LPL protein in the heart of CII mice. Although total LPL measured by immunoblot included extracellular as well as intracellular LPL in both active and inactive forms, heart is a tissue in which LPL has a high specific activity (26). Heart LPL activity was one of the significant predictors of plasma TG in mice with the human apoC-II transgene when analyzed by forward stepwise regression. In comparison, heart LPL activity was eliminated as a predictive variable in WT mouse data (data not shown). These results suggest that the amount of apoC-II affects the regulation of total LPL protein and the LPL activity at the endothelial surface in mouse heart. Moreover, as a major site of LPL-driven TG hydrolysis, the loss of ~30% of LPL activity in the heart could, in part, contribute to the accumulation of plasma TG observed in apoC-II transgenic mice. Although not significant, postheparin plasma LPL activity, a reflection of the total functional pool of LPL on the endothelium, in CII mice was less than half that measured in WT mice. As yet, the mechanism by which apoC-II could regulate LPL is unknown, but investigation into these interesting observations has the potential to provide more insight into the interaction and regulation of LPL by apoC-II.

In summary, overexpression of human LPL in skeletal muscle resulted in a reduction of plasma TG in apoC-II transgenic mice. This decrease was achieved in dose-dependent manner, and higher levels of LPL were necessary to overcome the inhibition attributed to excess apoC-II on the lipoprotein particle. These studies highlight the heart's contribution to whole body TG clearance and suggest the possibility that the human apoC-II transgene in mice may alter endogenous LPL expression/activity in the heart by some as yet unknown mechanism.

The authors gratefully acknowledge Dr. Jacqueline Etienne for generously providing the LPL antibody and Jennifer Yoon for her excellent assistance with the Western blots. This work was supported by National Institutes of Health Grant DK-26356 (to R.H.E.).

REFERENCES

1. Ferreira, L. D., L. K. Pulawa, D. R. Jensen, and R. H. Eckel. 2001. Overexpressing human lipoprotein lipase in mouse skeletal muscle is associated with insulin resistance. *Diabetes.* **50:** 1064–1068.
2. Jensen, D. R., I. R. Schlaepfer, C. L. Morin, D. S. Pennington, T. Marcell, S. M. Ammon, A. Gutierrez-Hartmann, and R. H. Eckel. 1997. Prevention of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. *Am. J. Physiol.* **273:** R683–R689.
3. Levak-Frank, S., H. Radner, A. Walsh, R. Stollberger, G. Knipping, G. Hoefler, W. Sattler, P. H. Weinstock, J. L. Breslow, and R. Zechner. 1995. Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. *J. Clin. Invest.* **96:** 976–986.
4. Yagyu, H., G. Chen, M. Yokoyama, K. Hirata, A. Augustus, Y. Kako, T. Seo, Y. Hu, E. P. Lutz, M. Merkel, et al. 2003. Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. *J. Clin. Invest.* **111:** 419–426.
5. Kim, J. K., J. J. Fillmore, Y. Chen, C. Yu, I. K. Moore, M. Pypaert, E. P. Lutz, Y. Kako, W. Velez-Carrasco, I. J. Goldberg, et al. 2001. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc. Natl. Acad. Sci. USA.* **98:** 7522–7527.
6. Breckenridge, W. C., J. A. Little, G. Steiner, A. Chow, and M. Poapst. 1978. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N. Engl. J. Med.* **298:** 1265–1273.
7. Cox, D. W., W. C. Breckenridge, and J. A. Little. 1978. Inheritance of apolipoprotein C-II deficiency with hypertriglyceridemia and pancreatitis. *N. Engl. J. Med.* **299:** 1421–1424.
8. Wang, C. S. 1991. Structure and functional properties of apolipoprotein C-II. *Prog. Lipid Res.* **30:** 253–258.
9. Fojo, S. S., and H. B. Brewer. 1992. Hypertriglyceridaemia due to genetic defects in lipoprotein lipase and apolipoprotein C-II. *J. Intern. Med.* **231:** 669–677.
10. Havel, R. J., C. J. Fielding, T. Olivecrona, V. G. Shore, P. E. Fielding, and T. Egelrud. 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoprotein lipase from different sources. *Biochemistry.* **12:** 1828–1833.
11. Shachter, N. S., T. Hayek, T. Leff, J. D. Smith, D. W. Rosenberg, A. Walsh, R. Ramakrishnan, I. J. Goldberg, H. N. Ginsberg, and J. L. Breslow. 1994. Overexpression of apolipoprotein CII causes hypertriglyceridemia in transgenic mice. *J. Clin. Invest.* **93:** 1683–1690.
12. Fornengo, P., A. Bruno, R. Gambino, M. Cassader, and G. Pagano. 2000. Resistant hypertriglyceridemia in a patient with high plasma levels of apolipoprotein CII. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2329–2339.
13. Poirier, P., T. Marcell, P. U. Huey, I. R. Schlaepfer, G. C. Owens, D. R. Jensen, and R. H. Eckel. 2000. Increased intracellular triglyceride in C2C12 muscle cells transfected with human lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **270:** 997–1001.
14. Eckel, R. H., P. A. Kern, C. N. Sadur, and T. J. Yost. 1986. Methods for studying lipoprotein lipase in human adipose tissue. *In* Methods in Diabetes Research. S. L. Pohl, W. L. Clarke, and J. Larner, editors. Wiley, New York. 259–273.
15. Belfrage, P., and M. Vaughan. 1969. Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
16. Glaser, D. S., T. J. Yost, and R. H. Eckel. 1992. Preheparin lipoprotein lipolytic activities: relationship to plasma lipoproteins and postheparin lipolytic activities. *J. Lipid Res.* **33:** 209–214.
17. Etienne, J., L. Noe, M. Rossignol, C. Arnaud, N. Vydelingum, and A. H. Kissebah. 1985. Antibody against rat adipose tissue lipoprotein lipase. *Biochim. Biophys. Acta.* **834:** 95–102.
18. Plump, A. S., J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin, and J. L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* **71:** 343–353.
19. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
20. Medh, J. D., G. L. Fry, S. L. Bowen, S. Ruben, H. Wong, and D. A. Chappell. 2000. Lipoprotein lipase- and hepatic triglyceride lipase-promoted very low density lipoprotein degradation proceeds via an apolipoprotein E-dependent mechanism. *J. Lipid Res.* **41:** 1858–1871.
21. Jong, M. C., V. E. Dahlmans, M. H. Hofker, and L. M. Havekes. 1997. Nascent very-low-density lipoprotein triacylglycerol hydrolysis by lipoprotein lipase is inhibited by apolipoprotein E in a dose-dependent manner. *Biochem. J.* **328:** 745–750.
22. Enerback, S., and J. M. Gimble. 1993. Lipoprotein lipase gene expression: physiological regulators at the transcriptional and post-transcriptional level. *Biochim. Biophys. Acta.* **1169:** 107–125.
23. Augustus, A. S., Y. Kako, H. Yagyu, and I. J. Goldberg. 2003. Routes of FA delivery to cardiac muscle: modulation of lipoprotein lipolysis alters uptake of TG-derived FA. *Am. J. Physiol. Endocrinol. Metab.* **284:** E331–E339.
24. Augustus, A., H. Yagyu, G. Haemmerle, A. Bensadoun, R. K. Vikramadithyan, S. Y. Park, J. K. Kim, R. Zechner, and I. J. Goldberg. 2004. Cardiac-specific knock-out of lipoprotein lipase alters plasma lipoprotein triglyceride metabolism and cardiac gene expression. *J. Biol. Chem.* **279:** 25050–25057.
25. Levak-Frank, S., W. Hofmann, P. H. Weinstock, H. Radner, W. Sattler, J. L. Breslow, and R. Zechner. 1999. Induced mutant mouse lines that express lipoprotein lipase in cardiac muscle, but not in skeletal muscle and adipose tissue, have normal plasma triglyceride and high-density lipoprotein-cholesterol levels. *Proc. Natl. Acad. Sci. USA.* **96:** 3165–3170.
26. Bergo, M., G. Olivecrona, and T. Olivecrona. 1996. Forms of lipoprotein lipase in rat tissues: in adipose tissue the proportion of inactive lipase increases on fasting. *Biochem. J.* **313:** 893–898.