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Increasing Adipocyte Lipoprotein Lipase Improves Glucose Metabolism in High Fat Diet-induced Obesity*

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Background: Lipoprotein lipase regulates fat uptake into adipose tissue.
Results: A mouse model with increased adipose tissue lipoprotein lipase has improved glucose metabolism when challenged with a high fat diet.
Conclusion: Increasing adipose tissue lipoprotein lipase improves adipose tissue function.
Significance: Adipose tissue lipoprotein lipase protects against obesity-induced glucose and insulin intolerance.

Lipid accumulation in liver and skeletal muscle contributes to co-morbidities associated with diabetes and obesity. We made a transgenic mouse in which the adiponectin (*Adipoq*) promoter drives expression of lipoprotein lipase (*LPL*) in adipocytes to potentially increase adipose tissue lipid storage. These mice (*Adipoq-LPL*) have improved glucose and insulin tolerance as well as increased energy expenditure when challenged with a high fat diet (HFD). To identify the mechanism(s) involved, we determined whether the *Adipoq-LPL* mice diverted dietary lipid to adipose tissue to reduce peripheral lipotoxicity, but we found no evidence for this. Instead, characterization of the adipose tissue of the male mice after HFD challenge revealed that the mRNA levels of peroxisome proliferator-activated receptor-γ (*PPARγ*) and a number of PPARγ-regulated genes were higher in the epididymal fat pads of *Adipoq-LPL* mice than control mice. This included adiponectin, whose mRNA levels were increased, leading to increased adiponectin serum levels in the *Adipoq-LPL* mice. In many respects, the adipose phenotype of these animals resembles thiazolidinedione treatment except for one important difference, the *Adipoq-LPL* mice did not gain more fat mass on HFD than control mice and did not have increased expression of genes in adipose such as glycerol kinase, which are induced by high affinity PPAR agonists. Rather, there was selective induction of PPARγ-regulated genes such as adiponectin in the adipose of the *Adipoq-LPL* mice, suggesting that increasing adipose tissue LPL improves glucose metabolism in diet-induced obesity by improving the adipose tissue phenotype. *Adipoq-LPL* mice also have increased energy expenditure.

Lipoprotein lipase (LPL)[2] is a central enzyme in lipid metabolism and adipose biology. LPL is synthesized and secreted by adipocytes and muscle and is transported to the capillary endothelium where it hydrolyzes the triglyceride (TG) core of circulating very low density lipoprotein (VLDL) and chylomicrons. The hydrolysis products are fatty acids and monoacylglycerol, which are taken up by the tissue. Depending upon the circumstance, such as the fed or fasting state, LPL delivers fatty acids to adipose tissue for storage or to heart and skeletal muscle as a fuel source. Therefore, LPL activity is highly regulated in these tissues, acting as a "metabolic gatekeeper" (1) to ensure that fatty acids are delivered to the correct tissue depending upon the physiological state (2).

In obesity, adipose tissue lipid accumulates up to a point, and then lipid is stored in ectopic sites such as liver and muscle. This increases diacylglycerides (DAGs) and ceramides, which inhibit insulin receptor signaling in these tissues (3). The accumulation of these ectopic lipids is thus postulated to cause insulin resistance along with inflammation and endoplasmic reticulum stress (3–5). Consistent with its ability to promote ectopic lipid accumulation, tissue-specific transgenic expression of LPL in skeletal muscle and liver induces insulin resistance (6), and LPL deficiency in muscle protects against insulin resistance in that tissue (7). Adipose tissue protects against the accumulation of ectopic lipids in these peripheral tissues by storing fat as neutral lipid (triglyceride). Lipid diversion into adipose tissue is one of the mechanisms of insulin sensitization by thiazolidinediones (TZDs) (8, 9), and *LPL* is an important gene target of TZDs (10). As expected, when there is insufficient adipose to store lipid, such as in adipose-deficient mice and humans, insulin resistance develops (11–13). Together, these observations suggest that diversion of lipid from muscle and liver into adipose tissue is generally beneficial.

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[2] The abbreviations used are: LPL, lipoprotein lipase; AdipoQ, adiponectin; PPAR, peroxisome proliferator-activated receptor; DAG, diacylglyceride; HFD, high fat diet; TG, triglyceride; WAT, white adipose tissue; BAT, brown adipose tissue; TZD, thiazolidinedione; NEFA, nonesterified fatty acid; ANOVA, analysis of variance.

In addition to lipid diversion, another predicted effect of increased adipose LPL is stimulation of PPAR transcription factors by the free fatty acids generated by lipoprotein hydrolysis. Depending upon the cell type and PPAR transcription factor expressed, activation of PPAR can have important physiological consequences. For instance, in macrophages or endothelial cells, LPL hydrolysis of VLDL activates PPAR transcription factors and has anti-inflammatory effects (14, 15). In muscle, LPL stimulates PPARδ, regulating mitochondrial biogenesis (16). In adipose tissue, LPL is in a positive feedback loop in which LPL stimulates PPARγ, and the *LPL* gene is induced by PPARγ (10). Activation of PPARγ in adipose tissue could result in numerous physiological outcomes, including insulin sensitization.

We have made a transgenic mouse in which the adiponectin promoter drives the expression of LPL in adipocytes, and these mice (*Adipoq-LPL*) have improved glucose tolerance and insulin tolerance when challenged with a high fat diet. We evaluated whether this was due to reduction of ectopic lipids in skeletal muscle and liver and/or activation of PPARγ in adipose tissue. We found that the adiponectin promoter used only modestly increased adipose tissue *Lpl* over endogenous levels, which was insufficient to cause a redistribution of lipid. However, there was sufficient expression of the transgene to improve the white adipose phenotype by increasing PPARγ and PPARγ-regulated genes such as adiponectin, resulting in an improved phenotype after HFD challenge.

EXPERIMENTAL PROCEDURES

Animal Studies—All of the studies involving mice were approved by the University of Kentucky Institutional Animal Care and Use Committee. The mice were housed in standard conditions at 22 °C, with a 14-h light and a 10-h dark cycle, and *ad libitum* access to standard rodent diet (Teklad Global 18% protein rodent diet; 2018; Teklad, Madison, WI) and water. Mice were given high fat diet (60% kcal from fat; D12492; Research Diets, New Brunswick, NJ) at the indicated age. Body composition was determined with an EchoMRI system (Echo Medical Systems, Houston, TX). Mice were evaluated by indirect calorimetry (TSE Systems, Chesterfield, MO). Wild-type female C57B6/J mice (The Jackson Laboratory, Bar Harbor, ME) were used for backcrossing.

Adipoq-LPL Transgenic Mouse—Human *LPL* was amplified by PCR using primers that added a 5′ HindIII site and a BamHI site in front of the stop codon using the *LPL* 3.6 plasmid (17) as a template. The PCR product was then subcloned into the HindIII and BamHI sites of pCMV-14 to add a C-terminal 3×FLAG epitope. Human *LPL*-3×FLAG was then amplified using a primer that added a ClaI site and 10 bases of its 5′-untranslated region, which corresponds to a strong Kozak sequence, and a 3′ primer that added an EcoRV site after the FLAG epitope stop codon. This PCR product was subcloned into the ClaI-EcoRV site of adiponectin 5.4, which is a construct that allows for very specific transgene expression in adipocytes using a modified adiponectin promoter, from Dr. Philip Scherer (18). The resultant plasmid was then digested with XhoI and KpnI to release the adiponectin 5.4 promoter-*hLPL*-3×FLAG construct. This was used by the transgenic mouse facility at the University of Kentucky to make *Adipoq-LPL* mice; there were several founder mice, but only one made detectable transgene protein. Studies in this work were from mice backcrossed 5–8 times into C57BL6 mice. Control mice were always littermate controls.

Glucose and Insulin Tolerance Tests—Blood glucose was measured using an AlphaTRAK glucometer (32004-02; Abbott). For glucose tolerance tests, mice were moved to the procedure room at least 4 h prior to the start of the procedure and fasted with free access to water. The animals were then weighed and intraperitoneally injected with D-(+)-glucose, 1 mg/kg body weight. Blood glucose levels were measured at baseline (immediately prior to injection), and at 30, 60, 90, and 180 min. For insulin tolerance tests, the mice were weighed, and 1.0–1.5 units Regular Human Insulin (Lilly) per kg of body weight was injected into the intraperitoneal cavity; blood glucose levels were determined at 0, 30, 60, 90, and 180 min.

Analysis of Serum—Adiponectin was quantified with an ELISA from Alpco (Salem, NH). Insulin and leptin were quantified using Milliplex Multiplex assays (Merck) and imaged on a Magpix system. Cholesterol was measured with a kit from Pointe Scientific (Brussels, Belgium); NEFA and triglycerides were determined with kits from Wako (Osaka, Japan). Serum was also fractionated on a fast protein liquid chromatography system to resolve lipoproteins as described (19). We then determined the area under the curve for the VLDL, LDL, and HDL peaks.

Histochemistry—Slides were deparaffinized and then incubated in Oil Red O (Sigma O0652; 0.3 g/ml in 60% isopropyl alcohol) for 30 min at room temperature. Slides were dipped in H_2O and coverslipped using a generous amount of aqueous mounting media.

Gene Expression—Gene expression was determined by real time reverse transcriptase polymerase chain reaction, as described previously (20), except that a Qiagen lipid kit (74804) was used. Standard curves were made from a pool of the cDNA, and gene expression was normalized to 18 S. This allows for the measurement of relative changes in gene expression within the indicated experiment. The primer sequences are in Table 1.

LPL FLAG Expression and Activity—Protein extracts were made in 50 mM Tris, pH 7.4, 500 mM NaCl, 1% Nonidet P-40, 20% glycerol, 1 mM DTT, 10 mM sodium pyrophosphate, 100 mM potassium fluoride, 1 mM EDTA, 1× Calbiochem protease inhibitors. The protein concentration was determined with a Bradford assay using BSA as a standard, and 30 μg was run on a 10% SDS-polyacrylamide gel. The proteins were then transferred to nitrocellulose, immunoblotted with the indicated antibody, and quantified with an Odyssey imaging system (LI-COR Biosciences, Lincoln, NE) as described (21). The antibody against FLAG (2368) was from Cell Signaling (Danvers MA), and the antibody against actin (A1978) was from Sigma. Heparin release from minced adipose tissue and measurement of LPL activity were done as described (22).

Energy Metabolism Studies—Mice were placed on a high fat diet for 12 weeks. The mice were then placed in acclimation chambers for 1 week and recorded in a TSE indirect calorimetry system for 1 week. Resting energy expenditure and net food intake were determined for the mice during the middle 3 days and analyzed by an ANCOVA-like model in which energy

TABLE 1
Primer sequences

Gene	Forward primer	Reverse primer
PPARα	ACA GGA GAG CAG GGA TTT GC	TAC CTA CGC TCA GCC CTC TT
PPARδ	GGC AGC CTC AAC ATG GAA TG	TCG AGC TTC ATG CGG ATT GT
PPARγ1/2	AGA CAG ACA TGA GCC TTC ACC	GGG TGG GAC TTT CCT GCT AAT
PPARγ2	TTC GCT GAT GCA CTG CCT AT	GGA ATG CGA GTG GTC TTC CA
Aco	TCG AAG CCA GCG TTA CGA G	GGT CTG CGA TGC CAA ATT CC
Cpt-1	TCC AGT TGG CTT ATC GTG GTG	TCC AGA GTC CGA TTG ATT TTT GC
Total *Lpl*	TTG TGG CCG CCC TGT	TCC TCC TCC ATC CAG TTG
Mouse *Lpl*	ACT CGC TCT CAG ATG CCC TA	TTG TGT TGC TTG CCA TCC T
CD36	GGC GTG GGT CTG AAG GAC TGG AA	GGA GGC ACG GGG TCT CAA CCA
Fabp4	ATG TGT GAT GCC TTT GTG GGA A	CTG CCA CTT TCC TTG TGG CAA
Atgl	GGT TAG AGT TGC TCA GCC GT	ACA TGA GGA GCG GAT GTG TG
Adiponectin	CAG TGG ATC TGA CGA CAC CAA	ACG TCA TCT TCG GCA TGA CTG
Ucp3	CCG ATT TCA AGC CAT GAT ACG C	CCT GGC GAT GGT TCT GTA GG
Scd-1	TTC TTG CGA TAC ACT CTG GTG C	CGG GAT TGA ATG TTC TTG TCG T
Pepck	AGG AGG GTG TCA TCC GCA AG	TGC TTT CGA TCC TGG CCA CA
Angplt4	CAT CCT GGG ACG AGA TGA ACT	TGA CAA GCG TTA CCA CAG GC
Sirt1	TGA TTG GCA CCG ATC CTC G	CCA CAG CGT CAT ATC ATC CAG
Ucp1	CAA AAA CAG AAG GAT TGC CGA AA	TCT TGG ACT GAG TCG TAG AGG
Pgc1α	TAT GGA GTG ACA TAG AGT GTG CT	CCA CTT CAA TCC ACC CAG AAAG
Glycerol kinase	TGA ACC TGA GGA TTT GTC AGC	CCA TGT GGA GTA ACG GAT TTC G
Oxidized *LDL* receptor	CCA CAG CAT GGA CGA ATT CA	AGC TTG CTT TGT GGC CTT CA
F4/80	TGA CTC ACC TTG TGG TCC TAA	CTT CCC AGA ATC CAG TCT TTC C
IL-1β	GCA ACT GTT CCT GAA CTC AAC T	ATC TTT TGG GGT CCG TCA ACT
Mcp-1	TTA AAA ACC TGG ATC GGA ACC AA	GCA TTA GCT TCA GAT TTA CGG GT
LPL transgene	AAC TCG ACG GTA TCG ATA GC	CTC CTC CAT CCA GTT GAT AA
Atp5a1	TCT CCA TGC CTC TAA CAC TCG	CCA GGT CAA CAG ACG TGT CAG
Cyto c	CCA AAT CTC CAC GGT CTG TTC	ATC AGG GTA TCC TCT CCC CAG
Sdha	GGA ACA CTC CAA AAA CAG ACC T	CCA CCA CTG GGT ATT GAG TAG AA
Vdac	CCC ACA TAC GCC GAT CTT GG	GTG GTT TCC GTG TTG GCA GA

expenditure was regressed against the final lean mass of the mice and the genotype.

Mass Spectroscopy—Skeletal muscle (gastrocnemius) and liver lipids were extracted with acidified organic solvents and analyzed by HPLC/ESI tandem mass spectroscopy as described (23–25). There were only five control mice in this experiment because one mouse was an outlier.

Statistics—Except where otherwise indicated, data for the two groups of mice were analyzed by an unpaired, two-tailed Student's *t* test. The weight gains on HFD and glucose and insulin tolerance tests were analyzed by repeated measures ANOVA. The resting energy expenditure and net food intake data were analyzed by an ANCOVA-like model with data related to genotype and lean mass, augmented by random effects to capture correlations among the repeated measurements on any particular animal. Resting respiratory exchange ratio data were analyzed by ANCOVA. Statistical significance was defined by a p value less than 0.05. Data analyses were carried out using Version 9.3 of SAS software, Graph Pad Prism 5, and JMP Version 10 SAS Institute.

RESULTS

Generation of Adipocyte-specific LPL-expressing Transgenic Mice—We made a transgenic mouse that expresses human *LPL* under the regulation of the adiponectin 5.4-kb promoter (Fig. 1*A*), which was previously shown to drive gene expression in adipocytes in a highly specific manner (18). There were several founder mice that expressed the transgene mRNA, but only one expressed detectable protein and was phenotyped. The transgene mRNA is expressed specifically in white adipose tissue (WAT) depots, but not other tissues (Fig. 1*B*), and the FLAG-tagged LPL transgene protein is detected at 56 kilodaltons in gonadal fat (Fig. 1*C*). We expected some transgene expression in brown adipose tissue (BAT) (18), but there was a very low

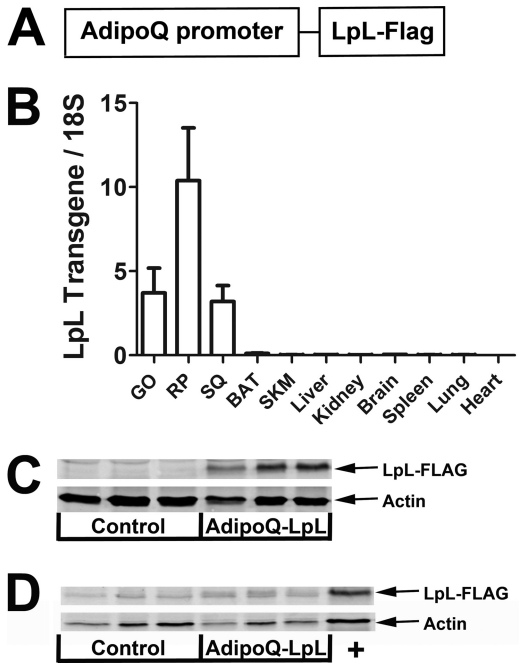

FIGURE 1. **Transgene design and expression in adipose tissue.** *A,* human LPL with a C-terminal 3×FLAG epitope tag was cloned into a vector containing the adiponectin 5.4-kb promoter. *B,* mRNA expression of the transgene in gonadal (*GO*), inguinal (*SQ*), retroperitoneal (*RP*), brown (*BAT*) adipose tissues; skeletal muscle (*SKM*); and the indicated tissues. *C,* protein expression of the transgene in gonadal fat of control and *Adipoq-LPL* male mice after high fat diet; the mass of the transgene was estimated using the Bio-Rad Precision Plus Protein Dual Color standard, which is not shown on the cropped image. *D,* protein expression of the transgene in BAT of control and *Adipoq-LPL* male mice after high fat diet; the positive control (+) is gonadal fat from an *Adipoq-LPL* mouse.

expression of the transgene in BAT at both the mRNA (Fig. 1*B*) and protein level (Fig. 1*D*; no expression detected above background). This low expression in BAT was not due to an unex-

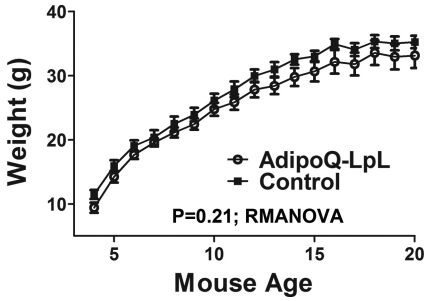

FIGURE 2. **Weight gain of control and *Adipoq-LPL* mice on HFD.** Mice were fed a HFD as described under "Experimental Procedures" and weighed weekly. The data are presented as means ± S.E., and the results of a repeated measures ANOVA are indicated.

TABLE 2

Serum and mouse characterization of male mice

Significant *p* values are in boldface type.

Parameter[a]	Control	*Adipoq-LPL*	*p*
Serum			
Fasting glucose (mg/dl)	203.4 ± 3.5	183.1 ± 5.1	**0.01**
NEFA (mmol/liter)	0.695 ± 0.011	0.670 ± 0.081	0.77
Total triglycerides (mg/dl)	59.42 ± 2.98	50.58 ± 4.84	0.15
Total cholesterol (mg/dl)	166.3 ± 7.2	129.5 ± 4.8	**0.01**
Insulin (ng/ml)	2.5 ± 0.6	2.8 ± 0.8	0.76
Leptin (ng/ml)	6.9 ± 2.9	7.5 ± 1.4	0.85
Adiponectin (μg/ml)[b]	17.0 ± 1.1	20.9 ± 0.7	**0.03**
Mouse characterization			
Weight at end of study	35.2 ± 1.0	33.1 ± 2.0	0.36
Gonadal fat pad mass	1.04 ± 0.04	1.01 ± 0.04	0.87
Fat mass (ECHO MRI)	13.2 ± 0.9	11.5 ± 1.2	0.29
Lean mass (ECHO MRI)	18.9 ± 0.8	18.8 ± 0.7	0.92
% fat (mean)	41 ± 2	37 ± 2	0.23

[a] *Adipoq-LPL* transgenic male mice and their littermate controls were characterized in a 19-week high fat (60% cal fat) feeding study ($n = 6$ control and $n = 6$ *Adipoq-LPL* per group). Data are represented as means ± S.E.

[b] $n = 4$ per group because serum was depleted for two mice in each group.

pected repression of the adiponectin promoter, which drives the transgene in the *Adipoq-LPL* mice, because adiponectin mRNA expression was similar in control and *Adipoq-LPL* BAT (see BAT gene expression below). Thus, the AdipoQ-LPL transgene is expressed in predominantly WAT depots.

Glucose and Insulin Tolerance—We hypothesized that the *Adipoq-LPL* mice would have an improved metabolic phenotype when challenged with a HFD. When challenged with a HFD (60% kcal fat), male *Adipoq-LPL* transgenic mice gained slightly less weight than their littermate controls (Fig. 2), but there was no statistically significant difference in weight gain when analyzed by repeated measures ANOVA (Fig. 2, $p = 0.21$) or in weight at the end of the study (Table 2; $p = 0.36$). The male *Adipoq-LPL* transgenic mice had improved glucose (Fig. 3*A*, $p = 0.0028$) and insulin (Fig. 3*B*, $p = 0.028$) tolerance. Female *Adipoq-LPL* mice had a trend for improved glucose tolerance (Fig. 3*C*, $p = 0.06$) but not improved insulin tolerance (Fig. 3*D*, $p = 0.52$). There was no statistically significant difference in glucose ($p = 0.99$) or insulin ($p = 0.28$) tolerance in male mice on a chow diet (Fig. 3, *E* and *F*).

Ectopic Lipid Distribution and Serum Lipids—One mechanism for the improved glucose tolerance could be that increased adipocyte LPL reduces ectopic lipid accumulation in liver and muscle by diverting it to fat. We stained liver and muscle from the HFD-fed male mice with Oil Red O and used mass spectroscopy to measure DAGs, TGs, and ceramides. As shown in Fig. 4, there was no qualitative difference in Oil Red O staining between the control and *Adipoq-LPL* mice in skeletal muscle (Fig. 4*A*) or in liver (Fig. 4*B*). Consistent with this, there was also no reduction in triglycerides, DAGs, or ceramides in these tissues in the *Adipoq-LPL* mice as measured by mass spectroscopy (Table 3), and the *Adipoq-LPL* mice in fact had an increase in liver TGs. There was also no evidence of increased fat mass or gonadal fat pad weights in the *Adipoq-LPL* mice after challenge with HFD (Table 2). Furthermore, serum NEFA and triglyceride levels were not significantly reduced in the *Adipoq-LPL* mice. Together, these results suggest that peripheral tissues are not protected from lipid accumulation in the *Adipoq-LPL* mice. Interestingly, the *Adipoq-LPL* mice had lower serum cholesterol levels (Table 2) and lower HDL ($p < 0.05$) and LDL ($p < 0.05$) when the sera were resolved by size exclusion chromatography (Fig. 5*A*). We also observed that triglycerides were reduced in the VLDL fraction (Fig. 5*B*, $p < 0.05$). Together, these data suggest that the *Adipoq-LPL* male mice have reduced lipoprotein levels, but this was not sufficient to protect peripheral tissues from lipid accumulation.

Improved Adipose Tissue Phenotype—An alternative mechanism of improved insulin sensitivity would be that increased adipocyte LPL stimulates PPAR transcription factors to improve adipose tissue function. Therefore, we measured the expression of PPAR transcription factors and well characterized genes that are PPAR-regulated in epididymal adipose tissue. We found that PPARγ1/2, PPARγ2, and several known genes regulated by PPARγ (*CD36, Adipoq*, and *Fabp4*) were all higher in the epididymal fat of the *Adipoq-LPL* mice than control mice after a high fat diet ($p < 0.05$; Table 4). Endogenous *Lpl*, which is also PPARγ-regulated, was induced 1.3-fold, and when we measured total *Lpl* by using primers that reacted with both human and mouse *Lpl*, there was only a 1.6-fold induction; thus, the adiponectin promoter only modestly increased total *Lpl* mRNA, which could explain why the peripheral tissues were not protected from ectopic lipid accumulation (see above). We measured heparin releasable LPL activity and found that there was an increase in the *Adipoq-LPL* mice, but it was not significant (Fig. 6, $p = 0.13$).

Interestingly, neither PPARα- nor the PPARα-regulated genes *Aco*, *Cpt-1*, and *Ucp-1* were up-regulated in the *Adipoq-LPL* mice (Table 4). *PPARδ* was induced, but *Fiaf* (*Angplt4*) and *Sirt1*, which are regulated by PPARδ, were not induced (Table 4). The expression of a number of mitochondrial genes was not altered (Table 4). Finally, a number of inflammatory genes *(IL-1β, MCP-1,* and *F4/80*) was reduced in the *Adipoq-LPL* male mice, but this did not reach significance (Table 4). Thus, the epididymal fat pads of the male *Adipoq-LPL* mice had a pattern of gene expression driven primarily by PPARγ, resulting in increased *Adipoq* and reduced inflammation. This pattern of gene expression was not observed in subcutaneous adipose tissue of the male mice, although *Adipoq* mRNA expression was higher in the SQ fat of the *Adipoq-LPL* mice (1.5-fold; $p = 0.113$). This together with the increased adiponectin expression in GO fat resulted in increased serum adiponectin in the male *Adipoq-LPL* mice (Table 2).

The female *Adipoq-LPL* mice did not have increased PPARγ or PPARγ-regulated gene (*Adipoq* and *CD36*) expression in their abdominal fat. In fact, endogenous *Lpl* was decreased in

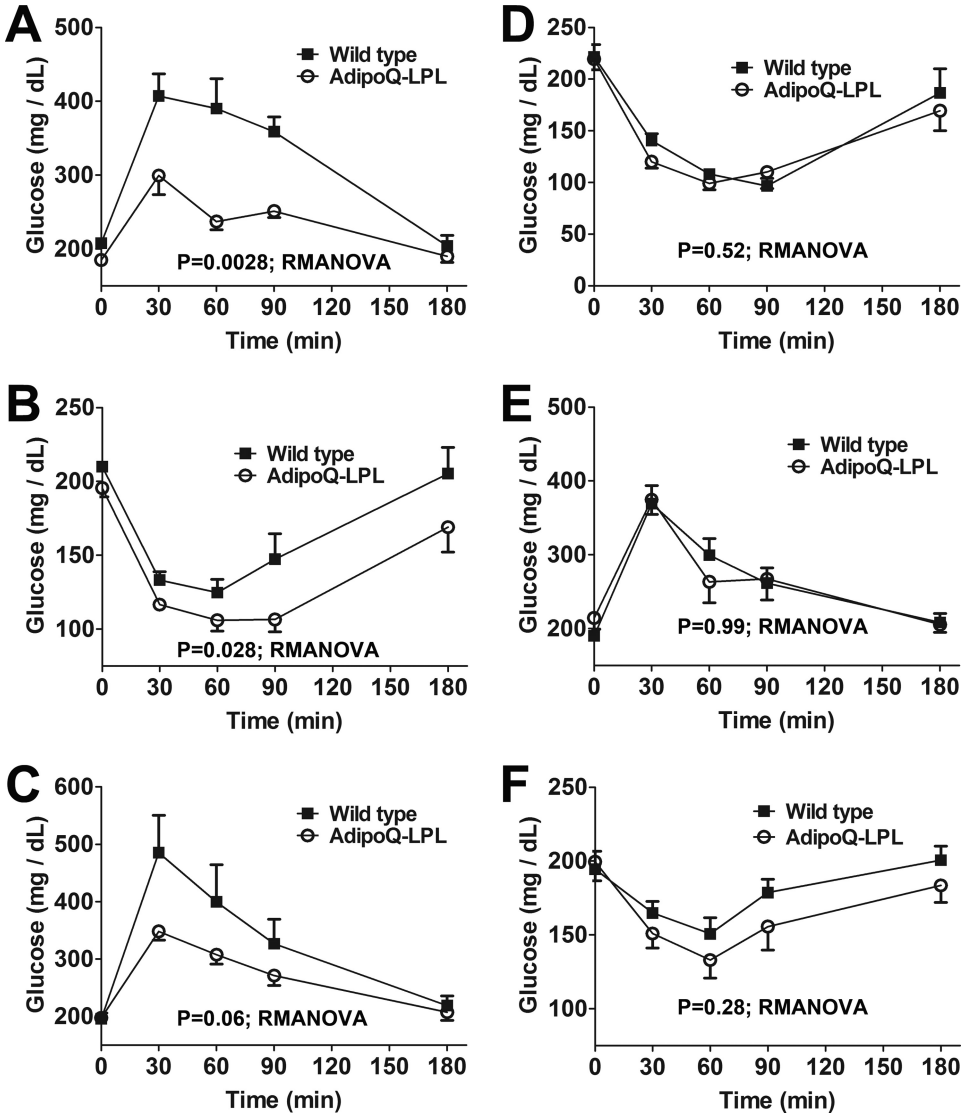

FIGURE 3. **Glucose and insulin tolerance of control and *AdipoQ-LPL* mice after HFD challenge.** The mice were fed a HFD as described under "Experimental Procedures." *A,* glucose tolerance test of male mice after HFD ($n = 6$ control, six *Adipoq-LPL*; $p = 0.0028$ by repeated measures ANOVA). *B,* insulin tolerance test on the same cohort of mice in *A* ($p = 0.028$ by repeated measures ANOVA). *C,* glucose tolerance test of female mice after HFD ($n = 6$ control, eight *Adipoq-LPL*; $p = 0.06$ by repeated measures ANOVA). *D,* insulin tolerance on the same cohort of female mice in *C*. *E,* glucose tolerance test of male mice on chow diet ($n = 8$ control, eight *Adipoq-LPL*; $p = 0.99$ by repeated measures ANOVA). *F,* insulin tolerance test on the same cohort of mice in *E* ($p = 0.28$ by repeated measures ANOVA). The data are presented as means ± S.E., and the results of a repeated measures ANOVA are indicated.

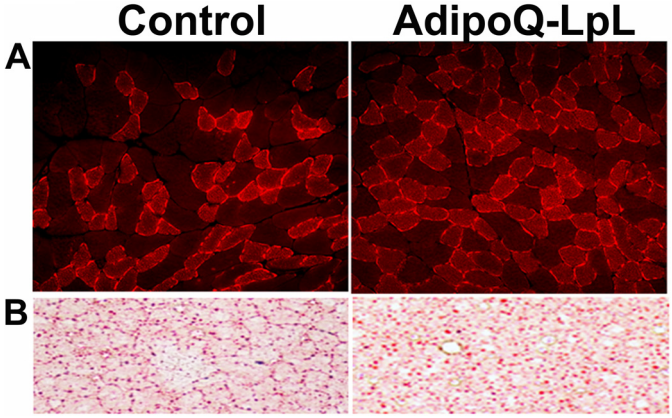

FIGURE 4. **Ectopic lipids in control and *AdipoQ-LPL* mice after HFD challenge.** Muscle (*A*) and liver (*B*) of control and *Adipoq-LPL* male mice from Fig. 2 were stained with Oil Red O as described under "Experimental Procedures."

female *Adipoq-LPL* mice (Table 5). However, there were trends for decreased inflammatory gene expression, including *IL-1β* and *MCP-1* ($p = 0.08$; Table 5), which were similar to the male mice. Also, there was no difference in the fat or lean mass of the control and *Adipoq-LPL* female mice at the end of the study (Table 6). These results suggest a different mechanism for the improved glucose tolerance observed in female *Adipoq-LPL* mice.

Mechanism of Insulin Sensitization, Similar to TZD Treatment?—The increase in PPARγ-regulated genes observed in the *Adipoq-LPL* mice is similar to pioglitazone treatment (26). However, there was no increase in weight or adipose mass (an undesirable side effect of TZD treatment) of the mice (Table 2), which would be expected if LPL hydrolysis products stimulated PPARγ as potently as TZDs. Because the free fatty acids produced by LPL are likely weak PPARγ agonists (14), we

TABLE 3

Tissue lipids

Significant *p* values are in boldface type.

Lipid[a]	Wild type	*Adipoq-LPL* transgenic	*p*
Muscle			
TG	2.81 ± 0.73	1.97 ± 0.55	0.37
DAG	0.0036 ± 0.0012	0.0028 ± 0.0009	0.58
Ceramide	0.28 ± 0.04	0.40 ± 0.04	0.06
Liver			
TG	0.10 ± 0.02	0.34 ± 0.09	**0.04**
DAG	0.00016 ± 0.00003	0.00044 ± 0.00014	0.22
Ceramide	0.0059 ± 0.0005	0.0057 ± 0.006	0.88

[a] Tissues from male *Adipoq-LPL* transgenic mice and their littermate controls were extracted for lipids and the extracts analyzed by mass spectrometry for lipids from the liver and muscle as indicated after the 19-week high fat (60% kcal fat) feeding study ($n = 5$ control and $n = 6$ *Adipoq-LPL* per group). The data are means ± S.E. The values are picomoles/nmol total phosphate.

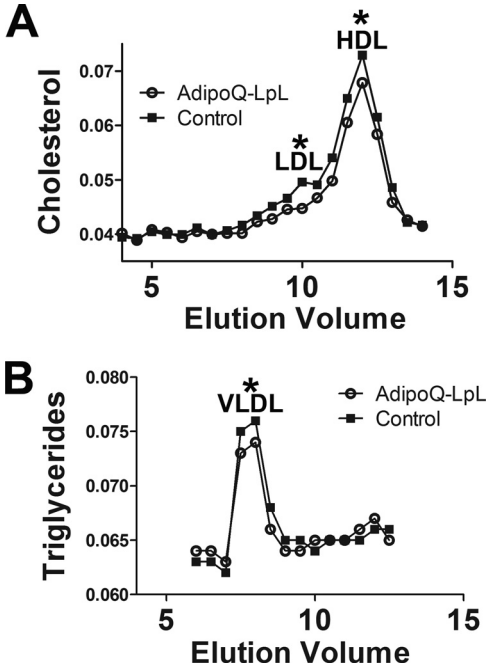

FIGURE 5. **FPLC size exclusion chromatography analysis of sera from control and *AdipoQ-LPL* mice.** The sera from the male mice in Fig. 2 were resolved by size exclusion chromatography, and cholesterol (*A*) and triglycerides (*B*) were measured as described under "Experimental Procedures"; the raw absorbance values are shown. *Asterisk* above the peaks indicates $p < 0.05$).

TABLE 4

Relative gene expression in perigonadal fat from male mice

Significant *p* values are in boldface type.

Gene[a]	Control	*Adipoq-LPL*	-Fold change Tg/Control	*p*
PPARα	1.0 ± 0.1	1.2 ± 0.0	1.2	0.47
PPARδ	0.9 ± 0.1	1.2 ± 0.1	1.4	**0.03**
PPARγ1/2	0.7 ± 0.0	1.4 ± 0.1	2.0	**<0.01**
PPARγ-2	0.9 ± 0.1	1.2 ± 0.0	1.4	**<0.01**
Aco	1.0 ± 0.1	1.0 ± 0.1	1.0	0.53
Cpt-1	1.0 ± 0.1	1.0 ± 0.2	1.0	0.38
Total *Lpl*[b]	0.8 ± 0.0	1.3 ± 0.2	1.6	**0.02**
Mouse *Lpl*[c]	0.9 ± 0.0	1.2 ± 0.1	1.3	**0.04**
CD36	0.8 ± 0.1	1.4 ± 0.4	1.9	**0.02**
Fabp4	0.8 ± 0.0	1.3 ± 0.0	1.6	**<0.01**
Atgl	0.9 ± 0.0	1.3 ± 0.0	1.4	0.08
Adiponectin	0.8 ± 0.1	1.4 ± 0.1	1.9	**<0.01**
Ucp3	1.0 ± 0.1	1.7 ± 0.1	1.7	0.08
Scd-1	0.8 ± 0.1	1.5 ± 0.2	1.8	**0.02**
Pepck	0.8 ± 0.2	1.6 ± 0.5	1.9	0.06
Angplt4	1.0 ± 0.1	1.2 ± 0.2	1.2	0.38
Sirt1	0.9 ± 0.1	1.2 ± 0.1	1.3	0.21
Ucp1	0.8 ± 0.1	0.7 ± 0.1	0.8	0.38
Pgc1α	1.2 ± 0.1	1.3 ± 0.1	1.0	0.91
Glycerol kinase	1.6 ± 0.0	1.0 ± 0.2	0.6	0.40
Oxidized *LDL* receptor	1.2 ± 0.0	1.0 ± 0.2	0.9	0.58
F4/80	1.5 ± 0.2	0.9 ± 0.0	0.6	0.18
IL-1β	2.7 ± 2.0	1.0 ± 0.0	0.5	0.37
Mcp-1	2.3 ± 2.1	1.0 ± 0.1	0.5	0.36
Atp5a1	0.9 ± 0.1	1.2 ± 0.1	1.3	0.20
Cyto c	1.0 ± 0.1	1.2 ± 0.0	1.2	0.40
Sdha	0.9 ± 0.1	1.2 ± 0.1	1.3	0.11
Vdac1	1.0 ± 0.1	1.1 ± 0.1	1.2	0.22

[a] Epididymal fat pads from *Adipoq-LPL* transgenic mice and their littermate controls were characterized after 19 weeks of a high fat (60% kcal fat) feeding study ($n = 6$ control; $n = 6$ *Adipoq-LPL*). The data are presented as means (arbitrary units) ± S.E.; the *p* value is for the difference in the mean.

[b] Data were measured with a primer set that recognizes human (*LPL* transgene) and mouse (endogenous) *Lpl*.

[c] Data were measured with a primer set that recognizes mouse (endogenous) Lpl 3′UTR.

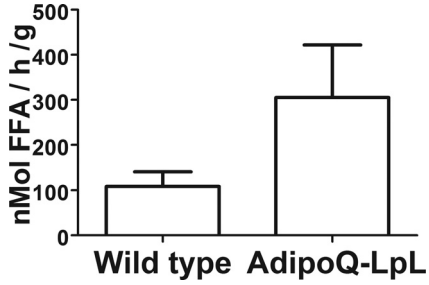

FIGURE 6. **Heparin-releasable LPL activity of epididymal fat of control and *AdipoQ-LPL* mice.** The heparin-releasable LPL activity in the epididymal fat of male mice fed an HFD was measured as described under "Experimental Procedures." The data are presented as means ± S.E.

determined whether the *Adipoq-LPL* mice had increased expression of genes known to require the high potency of TZDs, including glycerol kinase and the oxidized *LDL* receptor (27–29). These were not up-regulated in the *Adipoq-LPL* transgenic mice (Table 4), suggesting that LPL selectively modulates PPARγ to increase insulin sensitivity without side effects of full PPAR agonism.

Metabolic Phenotype—A recent study suggests that altering the tissue expression of LPL can affect energy balance (30). Therefore, we next characterized the mice by indirect calorimetry after 12 weeks of HFD challenge; the mice were maintained on the HFD while in the calorimetry chambers. The 24-h plots are shown in Fig. 7. The data were then filtered by time (between 9:00 a.m. and 6:00 p.m.) and activity (less than 150 counts for the previous 30 min) and then analyzed by ANCOVA to determine whether resting energy expenditure was affected by genotype (Fig. 8*A*). The *Adipoq-LPL* mice had increased resting energy expenditure (Fig. 8*A*, $p = 0.044$), which was not due to increased activity (Fig. 8*B*). The increased energy expenditure was also not due to either WAT browning (Table 4) or brown fat activation in the *Adipoq-LPL* mice because UCP1 and PGC1α were not induced there (Table 7). Also, PPAR transcription factors and adiponectin were not significantly induced in BAT in the *Adipoq-LPL* mice, suggesting that the LPL transgene effects are WAT-specific, consistent with its expression in that depot. The *Adipoq-LPL* mice weighed slightly more than the control mice (Fig. 8*C*, $p = 0.016$); however, the *Adipoq-LPL* mice ate more during the metabolic chamber study, which was significant when the data were analyzed by ANCOVA using lean mass as a covariate (Fig.

TABLE 5
Relative gene expression in perigonadal fat from female mice
Significant *p* values are in boldface type.

Gene[a]	Control	*AdipoQ-LpL*	Fold change TG/control	*p*
PPARα	1.5 ± 0.4	0.8 ± 0.1	0.6	0.06
PPARδ	1.5 ± 0.3	0.8 ± 0.1	0.6	**0.05**
PPARγ	.5 ± 0.2	0.3 ± 0.0	0.6	0.12
CD36	1.1 ± 0.1	1.0 ± 0.2	0.9	0.45
Fabp4	1.9 ± 0.5	0.8 ± 0.2	0.4	0.07
Adiponectin	1.2 ± 0.1	1.0 ± 0.2	0.8	0.33
F4/80	0.3 ± 0.0	0.3 ± 0.1	1.0	0.98
IL-1β	5.5 ± 1.2	3.0 ± 0.7	0.5	0.08
Mcp-1	2.1 ± 0.7	0.9 ± 0.3	0.4	0.08

[a] Perigonadal fat pads from female *Adipoq-LPL* transgenic mice and their littermate controls were characterized after 19 weeks of a high fat (60% kcal fat) feeding study ($n = 6$ control; $n = 8$ *Adipoq-LPL*). The data are presented as means (arbitrary units) ± S.E.; the *p* value is for the difference in the mean.

TABLE 6
Serum and mouse characterization of female mice

Parameter[a]	Control	*Adipoq-LPL*	*p*
Serum			
Fasting glucose (mg/dl)	197.4 ± 5.8	195.8 ± 9.9	0.88
Total triglycerides (mg/dl)	204.3 ± 30.0	161.8 ± 21.4	0.26
Total cholesterol (mg/dl)	153.0 ± 19.2	128.9 ± 12.0	0.33
Insulin (ng/ml)	0.53 ± 0.09	0.73 ± 0.17	0.37
Mouse characterization			
Weight at end of study	36.3 ± 1.8	34.5 ± 3.0	0.65
Fat mass (ECHO MRI)	16.9 ± 1.3	15.0 ± 2.3	0.53
Lean mass (ECHO MRI)	16.1 ± 0.6	16.1 ± 0.7	0.66
% Fat (mean)	50.9 ± 2.9	46.2 ± 3.4	0.27

[a] *Adipoq-LPL* female transgenic mice and their littermate controls were characterized in a 21-week high fat (60% cal fat) feeding study ($n = 6$ control and $n = 8$ *Adipoq-LPL* per group) that started at age 5 weeks. The data are presented as means ± S.E.; the *p* value is for the difference in the mean.

8*D*, $p = 0.001$). The resting respiratory exchange ratio was not different between genotypes ($p = 0.39$).

DISCUSSION

Because LPL generates free fatty acids for TG synthesis in adipocytes, an expected phenotype of the *Adipoq-LPL* mice would be increased adipose mass and relief of lipotoxicity in a high fat diet challenge. The *Adipoq-LPL* mice display no phenotype on chow and are protected against insulin and glucose intolerance when challenged with a high fat diet. The mechanism of improvement does not involve reduction of ectopic lipid accumulation in skeletal muscle or liver; plasma triglycerides and NEFAs were also not reduced in the *Adipoq-LPL* mice. Furthermore, the *Adipoq-LPL* mice do not accumulate more fat mass than the control mice; thus there is no evidence for lipid diversion, which is most likely explained by the modest increase in total LPL mRNA by the adiponectin promoter. Rather, the *Adipoq-LPL* mice have beneficial adipose tissue phenotype that is characterized by increased expression of adiponectin and reduced adipose inflammation. The gene expression profile of the epididymal fat suggests that the LPL transgene selectively modulates PPARγ activity. This modified adipose phenotype produces a systemic improvement in metabolism when the mice are challenged with a high fat diet.

The results of this study suggest that increasing adipocyte LPL in WAT results in improved glucose and insulin tolerance in high fat diet-induced obesity. This is the opposite phenotype of transgenic mice in which LPL was specifically induced in muscle (muscle-LPL) or liver (liver-LPL), leading to insulin resistance in those tissues (6). In the muscle-LPL and liver-LPL models, the increased tissue lipid led to an increase in DAGs and ceramides in the muscle and liver, respectively. Thus, one mechanism that we evaluated was whether the *Adipoq-LPL* mice had reduced ectopic lipids. However, we found no difference in Oil Red O staining; TG, DAG, and ceramide levels as measured by mass spectroscopy; or plasma TG and NEFA. Consistent with this, there was no increase in fat mass or gonadal fat pad weight. The lack of lipid redistribution is likely due to the fact that although the transgene is readily detectable at both the message and protein level, it is only increasing the overall message level modestly over endogenous *Lpl* (Table 4), and we were not able to demonstrate a significant increase in heparin releasable activity (Fig. 6). Thus, our transgene expression is not high enough to cause ectopic lipid redistribution. Others have shown that increasing LPL globally in rabbits improves glucose metabolism by reducing serum free fatty acids (31).

Despite the fact that we did not see a lipid redistribution effect, we did observe reduced cholesterol. A similar observation was made by Levak-Frank *et al.* (32), who found that increasing muscle LPL expression increased HDL turnover. It will be interesting to explore the mechanism in the future in the *Adipoq-LPL* model because there have been different reports of the effect of increasing LPL on HDL in other models, with some models of LPL overexpression having no change in HDL (31, 33, 34) and one model reporting an increase in HDL (35).

To determine the mechanism for improved glucose metabolism, we evaluated whether the *Adipoq-LPL* transgene could improve the WAT phenotype next. We extensively characterized gene expression in the WAT of the mice because LPL hydrolysis products could stimulate PPAR transcription factors. Activation of these transcription factors in adipocytes could promote insulin sensitivity by different mechanisms. Stimulation of PPARγ could promote insulin sensitivity by increasing adiponectin expression and/or other genes involved in glucose homeostasis (36). PPARα and PPARδ could promote insulin sensitivity by stimulating the β-oxidation of fat (37, 38). We observed that the *Adipoq-LPL* mice have increased gene expression of a subset of PPAR-regulated genes. Absent from this subset are genes that require the high affinity agonism of TZDs such as glycerol kinase (28, 29). Glycerol kinase produces glycerol 3-phosphate, which can then be used to re-esterify fatty acids for storage as triglyceride, promoting weight gain. Glycerol kinase is not up-regulated in the adipose of *Adipoq-LPL* mice, whereas it is up-regulated in the adipose of TZD-treated mice (29). Also, *Ucp1*, which is induced by TZDs, was not up-regulated in the *Adipoq-LPL* mice. However, *Fabp4* (AP2) does not require high affinity ligands for its expression to be induced (28), and *Fabp4* was up-regulated in the *Adipoq-LPL* transgenic male mice (Table 4). The results of our studies suggest that the induction of LPL in adipose tissue increases a select subset of endogenous PPARγ ligands to promote insulin sensitivity without the side effects of high affinity TZD agonists. This in part explains one of the remarkable features of the *Adipoq-LPL* mice; after high fat diet challenge, their fat mass is not higher than the controls, which is a predicted side effect of TZDs and an anticipated phenotype if increasing adipose LPL.

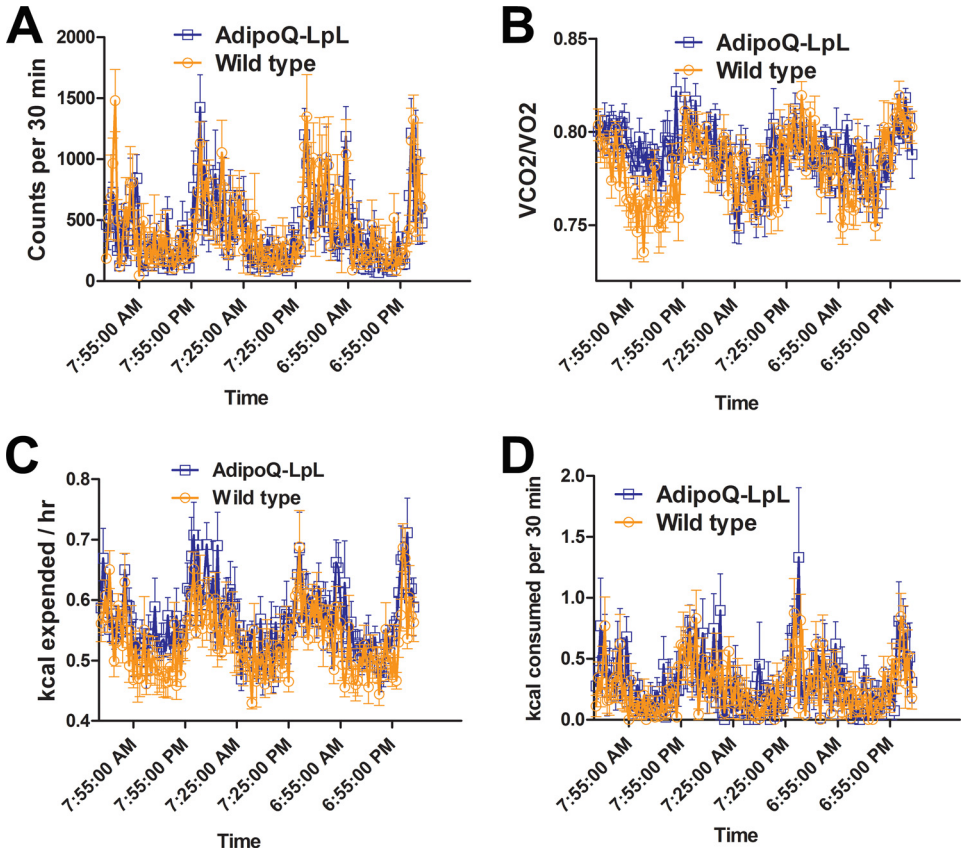

FIGURE 7. **24-h energy metabolism data from control and *Adipoq-LPL* mice during HFD challenge.** The mice were fed a high fat diet (60% calories from fat) for 12 weeks prior to placing them in calorimetry chambers; the data were analyzed as described under "Experimental Procedures." The mean data for the control and *Adipoq-LPL* mice are plotted as a function of time.

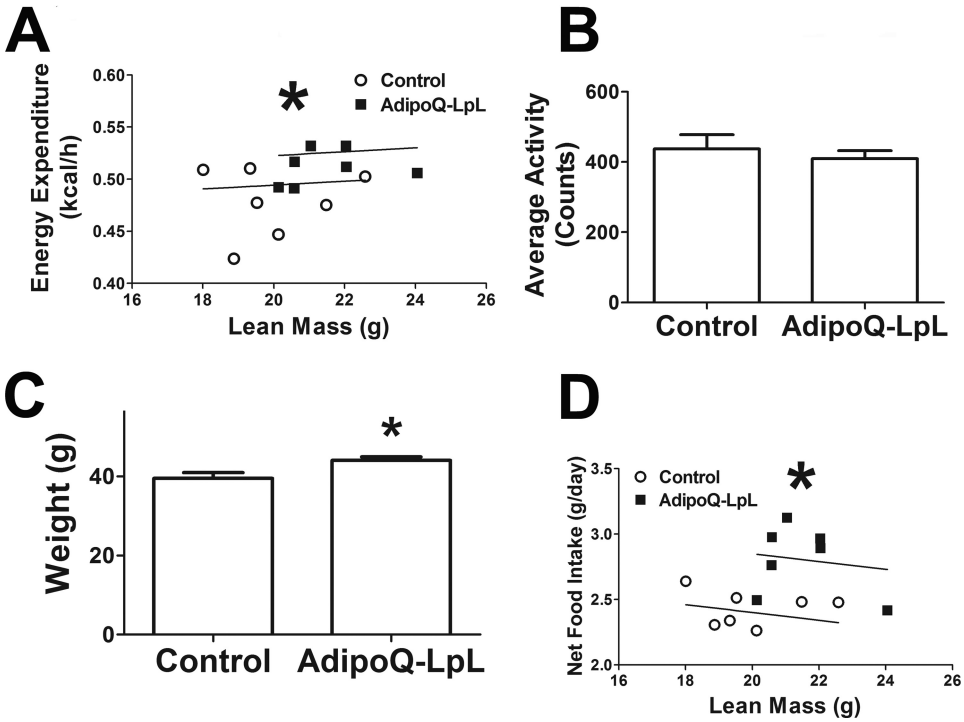

FIGURE 8. **Characterization of control and *AdipoQ-LPL* mice in calorimetry chambers during HFD challenge.** *A,* data from Fig. 7 were filtered by time and activity and analyzed with respect to the lean mass of the mice. Resting energy expenditure was plotted against post-lean mass; *, $p = 0.044$. *B,* average activity of the mice. *C,* weight of the mice after the study; *, $p = 0.016$. *D,* net food intake during the study; *, $p = 0.001$.

TABLE 7
Relative gene expression in BAT fat from male mice

Gene[a]	Control	*Adipoq-LPL*	-Fold change TG/Control	*p*
PPARα	1.2 ± 0.3	1.5 ± 0.6	1.3	0.62
PPARδ	1.0 ± 0.1	1.2 ± 0.2	1.2	0.39
PPARγ	0.2 ± 0.4	1.6 ± 0.6	1.4	0.50
Pgc1α	0.9 ± 0.3	1.4 ± 0.3	1.5	0.29
Adiponectin	1.0 ± 0.2	1.4 ± 0.4	1.4	0.34
Ucp1	1.6 ± 0.4	2.0 ± 0.7	1.3	0.60
Ucp3	0.9 ± 0.1	1.2 ± 0.2	1.2	0.29

[a] Epididymal fat pads from *Adipoq-LPL* transgenic mice and their littermate controls were characterized after 19 weeks of a high fat (60% kcal fat) feeding study ($n = 6$ control; $n = 6$ *Adipoq-LPL*). The data are presented as means (arbitrary units) ± S.E.; the p value is for the difference in the mean.

After 12 weeks of HFD challenge, the AdipoQ-LPL mice had higher energy expenditure than the controls. This response to the increased LPL could be the result of adipose tissue trying to defend its own mass (39) by increasing energy expenditure. The increased energy expenditure does not involve increased activity. It also does not involve browning of the WAT depots because *Pgc1α* and *Ucp-1* were not increased there. Nor does it involve BAT activation because *Pgc1α* and *Ucp1* are not increased in that depot. Also, unexpectedly, the LPL transgene was not expressed in BAT, suggesting that diversion of lipid to BAT is not occurring in the *Adipoq-LPL* mice. Of note, there was no difference in weight loss while in the acclimation chambers or weight gain while in the metabolic chambers. One possibility for the increase in energy expenditure would be that the improved WAT phenotype leads to this by an adipokine such as adiponectin, which causes increased energy expenditure (40, 41). However, the anti-inflammatory phenotype could be involved in this. This increase in energy expenditure is the opposite phenotype to knocking down LPL in neurons (30), but we did not find the transgene expressed in the brain, so the mechanism remains to be determined. In addition to the increase in energy expenditure, the *Adipoq-LPL* mice consumed more of the high fat diet. Thus, there is a futile cycle of increased energy expenditure and increased food intake. Nevertheless, this mouse model could thus be a useful tool to address the mechanism by which adipose defends its own mass by increasing energy expenditure in response to increased LPL in future studies.

In summary, increasing LPL in adipocytes improves adipose function and protects against glucose and insulin intolerance in diet-induced obesity. This occurs by the selective induction of PPARγ-regulated genes that lead to systemic improvements in metabolism and increased energy expenditure. Identifying these pathways and deciphering their contribution to regulation of metabolism is an important future goal. We are currently studying the acute response of this mouse model to high fat diet using microarrays to better understand the mechanism of the improved adipose phenotype.

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