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Down-regulation of lipoprotein lipase increases glucose uptake in L6 muscle cells

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ABSTRACT

Thiazolidinediones (TZDs) are synthetic hypoglycemic agents used to treat type 2 diabetes. TZDs target the peroxisome proliferator activated receptor-gamma (PPAR-γ) and improve systemic insulin sensitivity. The contributions of specific tissues to TZD action, or the downstream effects of PPAR-γ activation, are not very clear. We have used a rat skeletal muscle cell line (L6 cells) to demonstrate that TZDs directly target PPAR-γ in muscle cells. TZD treatment resulted in a significant repression of lipoprotein lipase (LPL) expression in L6 cells. This repression correlated with an increase in glucose uptake. Down-regulation of LPL message and protein levels using siRNA resulted in a similar increase in insulin-dependent glucose uptake. Thus, LPL down-regulation improved insulin sensitivity independent of TZDs. This finding provides a novel method for the management of insulin resistance.

Introduction

Peroxisome proliferator activated receptors (PPAR) are members of the nuclear-receptor gene super family of transcription factors. The PPAR-γ subtype is known to play a pivotal role in lipid and carbohydrate metabolism and is implicated in various metabolic and inflammatory disorders such as dyslipidemia, atherosclerosis, diabetes and obesity [1]. Natural ligands for PPAR-γ include long chain polyunsaturated fatty acids, lipid hydroperoxides, and various eicosanoids [2]. PPAR-γ are also high affinity targets of a class of synthetic agents called Thiazolidinediones (TZD) that improve insulin sensitivity, and are used for the management of type 2 diabetes [3].

Type 2 diabetes is characterized by insulin resistance, hyperinsulinemia and glucose intolerance. Administration of TZDs to diabetic or obese mice consistently reduces plasma glucose and insulin levels, improves insulin-stimulated glucose disposal and reduces hepatic glucose production [3]. In humans, treatment of type 2 diabetes with TZDs improves insulin sensitivity as evidenced by a decrease in fasting and postprandial plasma glucose, and near-normalization of hyperinsulinemia [3,4].

It is believed that the primary target of TZD administration is the adipose tissue [5]. PPAR-γ expression in adipose tissue is 10- to 30-fold higher than in muscle and liver. Adipocyte PPAR-γ activation is essential for adipogenesis and for maintaining insulin sensitivity [6]. It is unclear if TZDs act directly on muscle and liver PPAR-γ or if their effect in these tissues is secondary to adipose PPAR-γ activation [1]. It is significant that in spite of the low level of PPAR-γ expression in the muscle, transgenic mice with muscle-specific PPAR-γ failed to show TZD-mediated improvement in insulin sensitivity in the muscle [7].

A major effect of TZDs and PPAR-γ is to regulate lipid metabolism. TZDs promote storage of lipids in adipocytes, thereby depriving muscle and liver of triglycerides, which in turn improves their insulin sensitivity [6]. Lipoprotein lipase (LPL) plays a key role in regulating the entry of triglycerides into muscle and adipose tissue [8]. LPL is primarily synthesized in adipose tissue, muscle, and macrophages and it is regulated by various metabolic and endocrine stimuli in a tissue-specific manner. The promoter region of LPL has a functional PPAR response element (PPRE), causing TZDs to induce adipose tissue LPL expression [9].

We hypothesized that regulation of LPL in muscle may contrast that in the adipose tissue. Repression of muscle LPL by TZDs may explain their ability to restrict lipid accumulation in the muscle, and increase glucose utilization in the muscle [10]. In an attempt to clearly explain the relationship between TZD treatment, muscle LPL expression, and insulin sensitivity, we investigated the effect of PPAR-γ activation on LPL levels in L6 cells, a rat skeletal muscle cell line. We demonstrate a significant repression of LPL mRNA and protein expression in L6 cells treated with Ciglitazone. The decrease in LPL level coincided with an increase in insulin-stimulated glucose uptake. To ascertain that the increase in glucose

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uptake was directly related to LPL down-regulation, and not an independent effect of Ciglitazone treatment, we used LPL-specific siRNA to inhibit LPL expression, and showed a dependent increase in glucose uptake in siRNA-treated cells. Down-regulation of LPL also correlated with in increase in Glut4 transcript levels.

Methods

Cell culture and treatments. Rat skeletal muscle myoblasts (L6 cells) were obtained from ATCC and maintained in DMEM containing 10% FBS, splitting them at 50–60% confluency to prevent cell fusion. For experimentation, cells were plated in 12 well plates, and allowed to differentiate from myoblasts to myotubes by reducing the FBS content of the media to 2% for 2 days and subsequently to 0% for 2–4 days till several areas of fused myotubes were evident.

Fused multinucleated mytotubes were treated with 5 μM Troglitazone, 5 μM Ciglitazone or 5 μM of 15-deoxy-prostaglandin J_2 in DMEM media devoid of FBS. After 4 days, the cells were washed with PBS and either harvested in TRI reagent (Sigma Chemical Co.) for RNA isolation, or in lysis buffer (0.1% Triton-X 100 in 50 mM Tris–HCl, pH 8.0) for Western blot analysis, or cells were subjected to the glucose uptake assay.

LPL-specific siRNA duplexes were synthesized through Integrated DNA Technologies. The siRNA were 27 bp long with a two base 3′ overhang at the upstream end (Table 2). A mixture of rat LPL-specific siRNA oligonucleotides were also purchased from SantaCruz Biotechnology (SCBT). The siRNA stock solutions were reconstituted to 10–20 μM and they were used at a final concentration of 50–100 nM. Fully differentiated L6 myotubes were transfected with siRNA using the *Trans*IT-TKO Transfection reagent from *Mirus* or Lipofectamine 2000 (Invitrogen) in OptiMEM medium (Invitrogen) supplemented with 1% BSA. Seventy-two hours after treatment, cells were washed and harvested in TRI reagent or in cell lysis buffer, or they were subjected to the glucose uptake assay.

RNA isolation and RT-PCR. RNA was isolated using TRI reagent (Sigma) according to the manufacturer's protocol. The isolated total RNA was treated with RQ1 DNase to remove any contaminating genomic DNA and then purified using the 'RNA clean and concentrator' kit from Zymoresearch. Two to four micrograms of RNA per reaction was used to synthesize cDNA by reverse transcription (RT) using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT), dNTPs (0.44 mM), and either random hexamers or oligodT primers (all from Promega). The cDNA obtained as a product of the RT reaction was then used in PCR to check for relative mRNA levels.

Conventional PCR was performed using primer pairs shown (Table 1) or a nested primer pair set purchased from SCBT (sequences not provided by SCBT). The PCR amplicons were run on a 2% agarose gel and the DNA bands were quantified by ImageJ (NIH) analysis. The cDNA was also subjected to real-time quantitative PCR using a Smart cycler (Cepheid Inc.), SyBr green mix (Absolute QPCR mix Thermo Fisher Scientific), and primer pairs shown (Table 1). The resulting amplification and melt curves were analyzed to ensure specific PCR product. β-Actin was used as the house-keeping gene control for both conventional and quantitative PCR. The threshold cycle (C_T) values were used to calculate fold change in transcript levels using the $2^{-\Delta\Delta C_T}$ method [11] as follows:

$$\text{Fold change} = 2^{-(C_{T\ \text{target}} - C_{T\ \beta\text{-actin}})_{\text{siRNA}} - (C_{T\ \text{target}} - C_{T\ \beta\text{-actin}})_{\text{control}}}$$

Western blot analysis of LPL and β-actin. Since LPL is a secreted protein, its levels were quantified in the cell culture media by specific immuno-precipitation and Western blot analysis. Briefly, anti-LPL IgY (2 μg/mL) was incubated with goat anti-chicken IgY-agarose in immuno-precipitation (IP) buffer (20% glycerol, 0.15 M NaCl and 10 mM phosphate, pH 7.0) at 4 °C overnight. After washing away un-associated IgY, 100 μL of a 50% suspension of the agarose beads in IP buffer was mixed with 1 mL of conditioned media, collected from a culture of L6 cells treated as above. After incubation at 4 °C for 2 h, the beads were washed several times with cold IP buffer and the adsorbed proteins were eluted with 50 μL of 1× Lammelis buffer. Equal volumes of immunoprecipitated samples were resolved by 10% SDS–PAGE and transferred onto polyvinylidine fluoride membrane (Immobilon™-P) in transfer buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.5). The blot was first incubated in blocking buffer (20 mM Tris, 150 mM NaCl, 2 mM $CaCl_2$, 1% gelatin, 0.05% Tween-20, pH 7.4) overnight at 37 °C and then probed with rabbit anti-bovine LPL antibody lab650 (kindly provided by Dr. P.-H. Iverius) followed by peroxidase-conjugated goat-anti-rabbit secondary antibody (SCBT). The Supersignal West Pico chemiluminescent substrate (Pierce) was used to detect the protein bands. Results of Western blot analysis were quantitated using ImageJ.

Table 1
PCR primer sequences.

Gene	Primer name	Sequence
LPL	Sense2	5′-GGAATGTATGAGAGTTGGGT-3′
	Antisense1	5′-CCAGCAGCATGGGCTCCAA-3′
	Antisense2	5′-GGGCTTCTGCATACTCAAAG-3′
β-Actin	Sense	5′-TCATGAAGTGTGACGTTGACATCCGT-3′
	Antisense	5′-CTTAGAAGCATTTGCGGTGCACGATG-3′
Glut4	Sense	5′-GGCTGAGCTGAAGGATGAGA-3′
	Antisense	5′-ACCACACCAGCTCCTATGGT-3′

Glucose uptake. L6 myotubes treated as above were washed with PBS and stimulated with or without 100 nM insulin (Humulin R, Lilly) for 3 h in serum-free media. They were then washed twice with HBS (140 mM NaCl, 5 mM KCl, 2.5 mM $MgSO_4$, 1 mM $CaCl_2$, 20 mM HEPES, 0.5% BSA) and treated with Transport Solution (0.5 μCi/mL ^{3}H-2-deoxyglucose (specific activity 20 Ci/mmol) and 250 μM 2-deoxyglucose in HBS) for 6 min at room temperature or at 37 °C. Cells were then quickly washed with ice-cold saline containing 300 μM Phloretin, an inhibitor of membrane transport, and solubilized in 0.05 M NaOH. An aliquot of the lysate was analyzed for protein by the method of Lowry and the amount of radioactivity in the remainder was measured by scintillation counting.

Results

PPAR-γ activation represses LPL expression in L6 muscle cells

LPL expression is regulated in a tissue-specific manner with regulation in muscle and adipose tissues being in opposite directions [8]. Since adipose LPL is induced by TZDs, it was hypothesized that a direct repression of muscle LPL by TZDs may explain their ability to increase muscle insulin sensitivity.

The effect of PPAR-γ activation on LPL transcription was examined in L6 rat myotubes. There was no significant difference in cell morphology between control or treated cells. After 72 h of treatment, all cells were >90% differentiated with multinucleated myotubes. The level of β-actin transcript was used as a control for RNA loading on the gel, and remained unchanged after all treatments. Activation of PPAR-γ with 15d-PGJ_2, Troglitazone and Ciglitazone reduced LPL mRNA levels to 87%, 71% and 71% of untreated cells (Fig. 1A). Identical results were obtained after repeated experiments.

Activation of L6 cells with PPAR-γ ligands also reduced the amount of LPL protein secreted into the culture medium, as determined by Western blot analysis (Fig. 1B). Consistent with

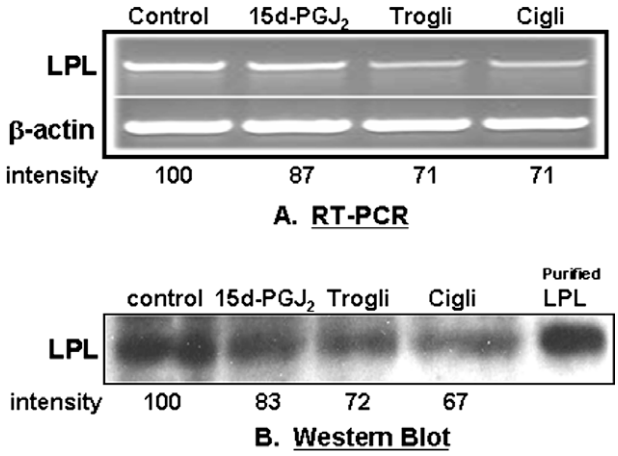


Fig. 1. Activation of PPAR-γ down-regulates LPL mRNA and protein levels in L6 rat skeletal muscle cells. L6 myoblasts were differentiated into myotubes as described in 'Methods'. Cells were incubated in media alone (control) or treated with 5 μM 15-deoxy-prostaglandin J_2 ((15d-PGJ_2), 5 μM Troglitazone (Trogli) or 5 μM Ciglitazone (Cigli) for 4 days. (A) Total RNA was isolated using TRI reagent, and subjected to RT-PCR using LPL primer pair sense2 and antisense2 as shown in Table 1. (B) LPL was immunoprecipitated from the cell culture media using anti-LPL IgY and anti-IgY-Sepharose and subjected to Western blot analysis using rabbit polyclonal anti-LPL antibody lab650. Purified bovine LPL (5 ng) is run alongside to verify the identity of the LPL band. (A and B) The relative intensity of each LPL band, determined by ImageJ analysis and corrected for β-actin, is shown below the lane.

the mRNA data, the TZDs were more potent at lowering LPL translation than 15d-PGJ_2. The LPL protein levels were 83%, 72% and 67% of control, respectively, for 15d-PGJ_2, Troglitazone and Ciglitazone-treated cells. LPL purified from bovine milk was run alongside to confirm the identity of immunoprecipitated LPL. For each experiment, the presence of equal cellular proteins was ascertained by analysis of β-actin protein levels in cell lysates (data not shown).

Ciglitazone increases glucose uptake in L6 cells

Next, it was investigated if a decrease in LPL transcript levels correlated with improved insulin sensitivity in L6 myotubes. The uptake of radiolabeled 2-deoxyglucose was used as a parameter for insulin sensitivity. Insulin stimulated glucose uptake 2.1-fold in control cells, whereas in Ciglitazone-treated cells, induction of glucose uptake by insulin was 3.1-fold (Fig. 2). Ciglitazone treatment resulted in a 1.6-fold increase in insulin stimulated glucose uptake. The results are corrected for cellular protein content which did not vary more than 1% between wells. Non-specific uptake of

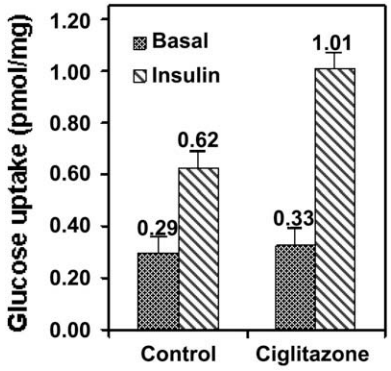


Fig. 2. Ciglitazone treatment induces insulin-stimulated glucose uptake in L6 myotubes. Differentiated L6 cells were treated with 5 μM Ciglitazone for 4 days. Cells were washed and incubated with (hatched bars, insulin) or without (solid bars, basal) 100 nM insulin for 3 h. The uptake of ^{3}H-2-deoxyglucose was measured for 6 min at room temperature as described in 'Methods'. Results of a representative experiment are shown and are averages of triplicate determinations.

glucose was measured in the presence of cytochalasin B, and was less than 10% of the total glucose uptake.

Down-regulation of LPL using siRNA

The repression of LPL expression and increased glucose uptake in L6 cells could be two independent effects of TZD treatment. In order to determine if there is a direct relationship between LPL expression level and insulin sensitivity, we down-regulated LPL expression in L6 cells using siRNA oligonucleotides (Table 2). Three different siRNA oligonucleotide treatments were used. The repression of LPL transcription was confirmed by conventional RT-PCR (Fig. 3A), and real-time quantitative PCR (Fig. 3B). Determination of the level of repression by ImageJ analysis of agarose gel images of conventional end-point RT-PCR showed repression of 32% using a combination of siRNA 908 and siRNA 2730, and a modest 18% reduction using siRNA R/H. When the siRNA available from Santa Cruz Biotechnology (SCBT) was used, a LPL-specific band was undetectable, suggesting complete knock-out. Since end-point PCR is not strictly quantitative, real-time quantitative PCR was performed using a Cepheid SmartCycler. The C_T values are shown in Fig. 3B. The fold change over control was calculated using the $2^{-\Delta\Delta C_T}$ method as follows:

$$\text{Fold change} = 2^{-(C_{T\ \text{target}} - C_{T\ \beta\text{-actin}})_{\text{siRNA}} - (C_{T\ \text{target}} - C_{T\ \beta\text{-actin}})_{\text{control}}}$$

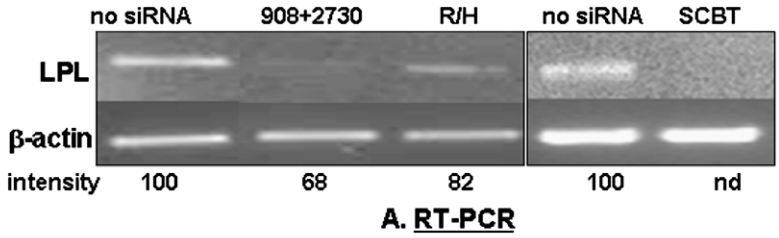
Using this method, LPL transcription was determined to be repressed to 21.6% and 33.2% of control, respectively, for treatments with siRNA 908 + 2730 or siRNA R/H. There was no specific amplification of LPL when L6 cells were treated with the SCBT siRNA indicating a complete knock-out of LPL mRNA. The silencing of LPL mRNA using siRNA also resulted in a ~30% reduction in LPL protein levels in the cell culture medium as determined by immuno-precipitation and Western blot analysis (Fig. 3C).

LPL down-regulation is co-incident with increased glucose uptake

In order to examine the relationship of LPL down-regulation with glucose uptake, we used L6 cells that had undergone several passages and were not highly sensitive to insulin stimulation. Untreated cells (no siRNA) showed only a 15–50% induction of glucose uptake upon insulin treatment (Fig. 4). Repression of LPL transcription resulted in significant increase in glucose uptake, both in the absence, and presence of insulin. Treatment with siRNA 908 + 2730 resulted in a 35% and 53% greater glucose uptake than control in basal and insulin-stimulated cells, respectively. The increase in glucose uptake after treatment with siRNA R/H was modest, probably due to the lower level of LPL repression (Fig. 3A) and therefore, the presence of functionally significant enzyme. On the other hand, the SCBT siRNA, which wiped out all detectable LPL

Table 2
LPL siRNA oligonucleotide sequences.

siRNA		Sequence
2730		5′-GCGCAAAGUACAAGUUUUAGAGCAG-3′ 3′-GACGCGUUUCAUGUUCAAAAUCUCGUC-5′
908		5′-GGAGAAGCCAUUCGUGUAAUUGCAG-3′ 3′-AACCUCUUCGGUAAGCACAUUAACGUC-5′
Rat/human (R/H)		5′-CCCUAAGGACCCCUGAAGACACAGC-3′ 3′-ACGGGAUUCCUGGGGACUUCUGUGUCG-5′
SCBT	798	5′-GGAUUUCGUAGAUGUCUUAtt-3′ 3′-ttCCU AAAGCAUCUACAGAAU-5′
	2384	5′-CAGGUGACAUAUGAAUUGAtt-3′ 3′-ttGUCCACUGUAUACUUAACU-5′
	2832	5′-GUACACUGUUGCGUGCAAAtt-3′ 3′-ttCAUGUGACAACGCACGUUU-5′



B. Q-RT-PCR C_T values			Fold change
	β-actin	LPL	
control	13.63	23.75, 23.75	1
908+2730	13.72	26.29, 25.81	0.216
R/H	13.62	25.53, 25.13	0.332
control	17.18	30.32, 30.80	1
SCBT	17.10	none detected	--

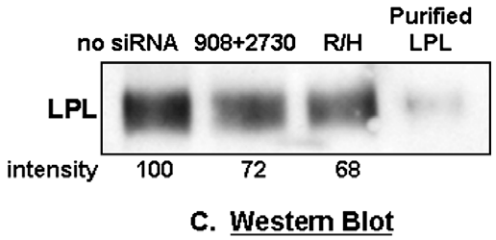


Fig. 3. Repression of LPL transcription and translation in L6 cells treated with LPL-specific siRNA oligonucleotides. L6 myotubes were transfected with different siRNA oligonucleotides shown in Table 2. The transfection reagent *Trans*IT-TKO was used to transfect a combination of 100 nM siRNA 908 and 100 nM siRNA 2730 (908 + 2730) or 100 nM siRNA R/H (R/H). Lipofectamine 2000 was used to transfect 50 nM of a cocktail of siRNA oligonucleotides obtained from SCBT (Table 2). (A) Total RNA was isolated after incubation with siRNA for 72 h. Conventional RT-PCR amplification for LPL (left 3 lanes) was performed using primer pair sense2 and antisense2 (Table 1). A set of primers obtained from SCBT (sequences not supplied by SCBT) were used to perform a nested PCR for LPL amplification (right 2 lanes). β-Actin was amplified using primers listed in Table 1. The intensity for each LPL band, corrected for β-actin is shown below the lane. When the SCBT siRNA was used, there was no detectable (nd) band for LPL. (B) Quantitative real-time RT-PCR was performed on a Cepheid SmartCycler using primer pairs for either LPL (sense2 and antisense1) or β-actin. The threshold cycle (C_T) values (shown in table) were used to calculate the amount of LPL cDNA in siRNA-treated cells relative to control using the $2^{-\Delta\Delta C_T}$ method as follows: Fold change $= 2^{-(C_T\ \text{target} - C_T\ \beta\text{-actin})_{\text{virus}} - (C_T\ \text{target} - C_T\ \beta\text{-actin})_{\text{control}}}$. There was no specific amplification of LPL after treatment with SCBT siRNA. (C) Western blot analysis of LPL protein from culture media of siRNA-treated L6 cells was performed as described in Fig. 1.

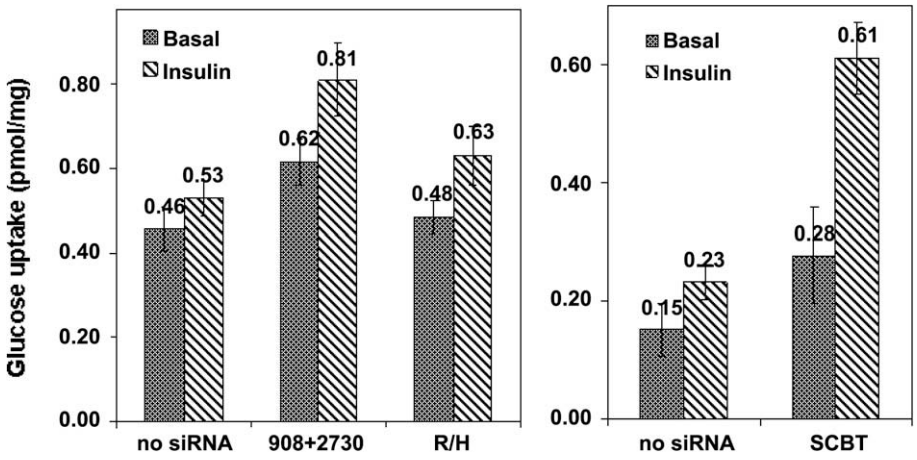


Fig. 4. Down-regulation of LPL expression induces glucose uptake in L6 myotubes. Differentiated L6 cells were treated with LPL-specific siRNA oligonucleotides for 3 days as described in Fig. 3. Cells were washed and incubated with (hatched bars, insulin) or without (solid bars, basal) 100 nM insulin for 3 h. The uptake of ^{3}H-2-deoxyglucose was measured for 6 min at 37 °C as described in 'Methods'. Results of a representative experiment are shown as averages of triplicate determinations.

transcript, resulted in a 87% and 165% induction in glucose uptake in basal and insulin-stimulated L6 cells, respectively. Insulin sensitivity, when measured as an increase in glucose uptake after insulin treatment in the same population of cells, was 31% in both, siRNA-908 + 2730, and siRNA R/H-treated cells. In siRNA SCBT-treated cells, insulin sensitivity was 118%. Our results clearly indicate that the extent of glucose uptake is inversely related to the amount of LPL transcript.

Since insulin-stimulated glucose uptake is mediated by the glucose transporter Glut4, we compared Glut4 transcript levels in normal and LPL-deficient L6 cells. Quantitative RT-PCR data consistently showed a lower C_T values for Glut4 in LPL siRNA-transfected cells compared to control cells (Table 3). Using the $2^{-\Delta\Delta C_T}$ method, the Glut4 transcript level was calculated to be 2- to 4-fold greater in LPL-deficient cells, in three independent experiments. Based on these data, it appears that a signaling cascade may link cellular LPL activity to Glut4 transcription and glucose uptake.

Discussion

Thiazolidinedione treatment in experimental animals and humans markedly improves whole-body insulin sensitivity. Thus, TZDs have been used for several years now to manage type 2 diabetes. While it is clear that TZDs target PPAR-γ to improve insulin sensitivity, the primary target tissues have not yet been unequivocally identified. Also, the post-receptor signaling pathways that lead to improved glucose uptake are not apparent. One working hypothesis for TZD action is that PPAR-γ stimulates adipogenesis and promotes

Table 3
Glut4 Q-RT-PCR C_T values.

	Expt 1		Expt 2		Expt 3	
	Control	SCBT siRNA	Control	SCBT siRNA	Control	R/H siRNA
β-Actin	17.18	17.10	18.55	18.24	15.82	15.14
Glut4	36.14	34.96	32.93	30.95	30.45	27.69
Fold change	1.00	2.14	1.00	3.18	1.00	4.22

the formation of insulin-sensitive adipocytes. Increased adiposity helps sequester fatty acids away from metabolic tissues such as liver and muscle and forces them to utilize glucose [10].

Studies in experimental animals have provided evidence for a relationship between PPAR-γ activity, adipogenesis and insulin sensitivity [12]. In lipoatrophic mice, a complete absence of adipocytes is responsible for insulin resistance which is not corrected by TZD administration. Obese diabetic mice are also insulin resistant due to too much adipose tissue, however, they are responsive to TZD treatment [12]. These results suggest that TZDs target adipocytes to improve insulin sensitivity.

Recent studies have questioned an exclusive role for adipose PPAR-γ in the insulin sensitizing function of TZDs. The muscle and liver are the most insulin-responsive tissues. Insulin resistance is characterized by unregulated gluconeogenesis and glycogenolysis in the liver, and decreased glucose uptake in the muscle [6]. Since the muscle is normally responsible for clearance of more than 70% of plasma glucose, it is important to understand the regulation of insulin sensitivity in the muscle. Mice with muscle-specific deletion of PPAR-γ showed increased adiposity, whole body glucose intolerance and insulin resistance [7,13]. Treatment with TZDs significantly decreased plasma glucose, insulin, triglyceride and free fatty acid levels in muscle PPAR-γ KO mice, suggesting activation of liver and adipose PPAR-γ. However, insulin-stimulated glucose disposal rates, measured using the hyperinsulinemic–euglycemic clamp technique did not respond to TZD treatment indicating a role for muscle PPAR-γ in TZD-mediated insulin sensitization [7]. Clearly, the mechanisms of TZD action are quite complex and there is evidence for cross-talk between adipose, muscle, and liver. Thus, in order to unambiguously understand the role of muscle as a target tissue and as an effector organ of TZD action, it is preferable to work with cultured cells.

Very few studies have directly investigated the effect and mechanism of TZD action in cultured muscle cells. An early report by Ciaraldi et al. showed that CS-045 (Troglitazone) increased insulin sensitivity in HepG2 (liver) and BC3H-1 (muscle) cells [14]. In this study, increased glycogen synthesis and decreased gluconeogenesis were used as indicators of insulin sensitivity. They did not see a change in basal or insulin-stimulated glucose transport in muscle cells. Yonemitsu et al. showed increased glucose uptake in L6 myotubes after 24 h exposure to Troglitazone; this increase was attributed to Glut4 translocation to the plasma membrane rather than an increase in Glut4 expression levels [15]. Using a rat skeletal muscle cell line (L6 cells), we have demonstrated here a direct relationship between PPAR-γ activation by Ciglitazone and glucose uptake in muscle cells. We found that PPAR-γ activation resulted in a significant down-regulation of LPL transcript and protein levels.

A few studies have investigated the effect of muscle LPL on insulin sensitivity. In general, muscle LPL levels appear to correlate with insulin resistance [16]. Eckel and colleagues showed that mice with muscle-specific over-expression of LPL develop whole body and muscle-specific insulin resistance along with accumulation of triglycerides and fatty acid metabolites in muscle tissue [10]. Similarly, Kim et al. showed that muscle-specific lipoprotein lipase expression impaired both metabolic and signaling functions of insulin [17].

While most studies have focused on over-expression of muscle LPL, we have shown, for the first time, that down-regulation of LPL in isolated muscle cells increases their insulin sensitivity. Additionally, quantitative RT-PCR data suggest that down-regulation of LPL induces Glut4 transcription in these cells. It is likely that Glut4 translocation, or stability, may also contribute to increased glucose uptake. Additional signaling events such as protein phosphorylation, or inter-protein interactions, may be involved in linking LPL down-regulation to the end function of increased glucose uptake. Other genes involved in lipid and carbohydrate metabolism are known targets of TZDs. It will be interesting to determine if repression of LPL can mimic some of these effects of TZDs. In view of recent concerns about TZD's adverse effects on cardiac health [18], down-regulation of LPL may provide a novel TZD-independent approach to improving insulin sensitivity in the muscle.

Acknowledgments

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