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Lipoprotein lipase mRNA in white adipose tissue but not in skeletal muscle is increased by pioglitazone through PPAR-γ

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Abstract

Lipoprotein lipase (LPL), a key enzyme for triglyceride hydrolysis, is an insulin-dependent enzyme and mainly synthesized in white adipose tissue (WAT) and skeletal muscles (SM). To explore how pioglitazone, an enhancer of insulin action, affects LPL synthesis, we examined the effect of pioglitazone on LPL mRNA levels in WAT or SM of brown adipose tissue (BAT)-deficient mice, which develop insulin resistance and hypertriglyceridemia. Both LPL mRNA of WAT and SM were halved in BAT-deficient mice. Pioglitazone increased LPL mRNA in WAT by 8-fold, which was substantially associated with a 4-fold increase of peroxisome proliferator activated receptor (PPAR)-γ mRNA ($r = 0.97,\ p < 0.0001$), whereas pioglitazone did not affect LPL mRNA in SM. These results suggest that pioglitazone exclusively increases LPL production in WAT via stimulation of PPAR-γ synthesis. Since pioglitazone does not affect LPL production in SM, this would contribute to prevent the development of insulin resistance due to lipotoxicity.

Keywords: Pioglitazone; Lipoprotein lipase; PPAR-γ; Brown adipose tissue-deficient mice; Triglyceride metabolism

Lipoprotein lipase (LPL) is a rate limiting enzyme for triglyceride (TG) hydrolysis that regulates the concentration of plasma TG [1]. LPL regulates the entry of fatty acids into adipose tissues and muscles [2,3]. Insulin plays an important role in the biosynthesis of this enzyme, thus LPL production and its lipolytic activity would be decreased in an insulin-resistant state [4,5]. However, it still remains largely unknown as to how insulin resistance affects LPL synthesis in adipose tissues and skeletal muscles. The effect of insulin on LPL synthesis and release is different in adipose tissues and skeletal muscles [6], which complicates this tissue.

Thiazolidinedione (TZD), an agonist for the nuclear hormone receptor, peroxisome proliferator activated receptor-gamma (PPAR-γ), is widely used as an effective therapy for type 2 diabetic patients with insulin resistance. TZD facilitates the differentiation of adipocytes through the action of PPAR-γ and generates small-sized adipocytes, which results in an improvement of insulin resistance [7]. PPAR-γ is highly expressed in white adipose tissue (WAT) and brown adipose tissue (BAT) [8–11], but it is not abundant in skeletal muscles. There are a number of studies demonstrating that TZD has a hypotriglyceridemic action in insulin-resistant state [12,13]. However, it remains unclear whether this is due to an increase of LPL biosynthesis. Even if TZD stimulates LPL synthesis, it remains unknown as to how PPAR-γ is involved.

Brown adipose tissue (BAT) is responsible for non-shivering thermogenesis, which is a major component of the energy expenditure to control body weight and fuel metabolism. Lowell et al. [14] created a transgenic mouse with ablation of BAT, archive by using regulatory elements of the uncoupling protein (UCP) 1 gene expressed specifically in brown adipocytes, to drive expression of diphtheria toxin A chain (DTA). These BAT-ablated transgenic mice (UCP–DTA) are

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characterized by hypothermia, reduced energy expenditure, obesity, hypertriglyceridemia, and impaired glucose tolerance associated with insulin resistance [14–16].

In this study, we investigated the mechanism of the TG-lowering effect of TZD, and especially tried to determine the effect of TZD on LPL synthesis in representative insulin-sensitive organs, i.e., WAT and skeletal muscles. We also examined the association between LPL and PPAR-γ. Since there is little BAT in adult humans [17], UCP–DTA mice appeared to be an useful model of adult humans with insulin resistance. In addition, this animal does not have BAT, an organ abundant in LPL and PPAR-γ, therefore, it is useful to investigate LPL and PPAR-γ in WAT.

Materials and methods

Animals. We used BAT-deficient mice (uncoupling protein-1 promoter-driven diphtheria toxin A, UCP–DTA), originally established by Flier's Laboratory [14]. The BAT-deficient mice (FVB background) were purchased from The Jackson Laboratory (Bar Harbor, ME) and the animals were mated in our laboratory. Male UCP–DTA mice were used in all the experiments. Male FVB/N mice were purchased from Saitama Experimental Animal Supply (Saitama, Japan). They were maintained on a 12-h light, 12-h dark cycle under controlled temperature and humidity, and had free access to water and food. UCP–DTA and FVB/N mice were used for the experiments at the age of 12–14 weeks. The UCP–DTA mice were fed standard mouse chow (Oriental Food, Tokyo) with or without 0.01% pioglitazone for 2 weeks. Pioglitazone (Actos) was a kind gift of Takeda Chemical Industries (Osaka, Japan). The FVB mice were fed ad libitum standard mouse chow. Food was removed at 9:00 a.m. on the day of the experiments and all experiments were carried out after a 5-h fast.

Triglyceride secretion rate. Triglyceride secretion rate (TGSR) was determined by measuring the increase in plasma TG concentration after an intravenous injection of Triton WR 1339 (Sigma Chemical, St. Louis, MO) (500 mg/kg body weight, 25% solution in saline). Mice were anesthetized with pentobarbital sodium, and blood was collected immediately before Triton WR 1339 injection and at 30, 60, and 90 min thereafter. Plasma TG concentration was found to increase linearly ($r > 0.98$) over the 90-min period in each mice. TGSR was calculated from the increment in TG concentration per minute multiplied by the plasma volume of mouse (estimated to be 0.035% of body weight (g)) and expressed in mg/min.

LPL activity in post-heparin plasma. Post-heparin blood samples were obtained at 10 min after the injection of heparin (1000 U/kg) into the femoral vein, and LPL activity was determined as the rate of release of radiolabeled fatty acids from a [^{14}C]triolein emulsion in Triton X-100 according to the method of Krauss et al. [18].

Subcloning of PPAR-γ cDNA fragment by RT-PCR. PPAR-γ cDNA was synthesized from total RNA in WAT because PPAR-γ mRNA is abundantly expressed in WAT. Total RNA (0.8 μg) from WAT of Sprague–Dawley rat was reverse-transcribed by random priming with Moloney murine leukemia virus reverse transcriptase (Life Technologies, Rockville, MD). And then the first-strand cDNA was utilized for polymerase chain reaction using specific oligonucleotide primers (5′-TGACATCAAACCCTTTACCACG-3′ and 5′-CTGTGACAATCTGCCTGAGGTC-3′, GenBank Accession No. Y12882) and AmpliTaq Gold (Perkin–Elmer, Foster, CA). The resulting 1247-bp fragment was cloned into TA-cloning vector, pGEM-T Easy (Promega, Madison, WI). The nucleotide sequence of the PPAR-γ insert in pGEM-T Easy was confirmed by an Automatic DNA Sequencer (Shimadzu, Kyoto, Japan). The plasmid carrying PPAR-γ insert was digested with *Eco*RI to obtain the PPAR-γ cDNA fragment.

RNA preparation. Total RNA was prepared from epididymal WAT and lower limb skeletal muscles of each animal using TRIzol reagent (Life Technologies) according to the protocol provided by the manufacturer. The concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm. The quality of the RNA was confirmed by ethidium bromide staining of 18S and 28S ribosomal RNA after electrophoresis on 1% agarose gel containing 6% formaldehyde.

RNA quantification by Northern blot analysis. Total RNA was loaded at 15 μg per lane onto 1% agarose gel containing 6% formaldehyde and transferred onto a nylon membrane (Hybond N$^+$, Amersham, Arlington Heights, IL). Then, each membrane was stained with methylene blue and then equivalent loading was verified by the density of both 18S and 28S rRNA bands visualized by the methylene blue staining. After pre-hybridization for 4 h, membranes were hybridized with [α-^{32}P]dCTP-labeled cDNA probe (Megaprime, Amersham) for mouse LPL (a gift of Dr. R. Zechner, Karl-Franzens University, Graz, Austria [19]), mouse UCP2 (a gift of Dr. D. Ricquier, Centre de Recherche sur l'Endocrinologie, Meudon, France [20]), and rat PPAR-γ at 42 °C for 16 h. The membranes were washed twice for 30 min at 60 °C in 2× SSC, 1× SDS, and 0.2% sodium pyrophosphate, and then washed for 10 min at room temperature in 0.2× SSC and 0.1× SDS. Signals were analyzed and quantified using the Fujix BAS2000 Bioimaging Analyzer (Fuji Photo Film, Tokyo, Japan) and then exposed to X-ray films (Fuji Photo Film) at −80 °C, using an intensifying screen.

Biochemical measurements. Plasma glucose levels were determined by the glucose oxidase method (Glucose B-test, Wako Pure Pharmaceutical Industries, Osaka). Plasma immunoreactive insulin concentrations and plasma leptin concentrations were determined using the specific ELISA Kits (Morinaga, Tokyo). Plasma triglyceride concentration was determined by an enzymatic method using a kit (triglyceride-G, Wako Pure Pharmaceutical).

Statistical analysis. Data are expressed as means ± SE. The significance of the difference in mean values of metabolic parameters between UCP–DTA and FVB mice was evaluated by unpaired Student's t test. The significance of the difference in mean values of metabolic parameters in UCP–DTA mice between pre- and post-treatment with pioglitazone was evaluated by paired Student's t test. Differences in gene expression between UCP–DTA and FVB/N and pre- and post-treated UCP–DTA mice were analyzed for statistical significance by unpaired Student's t test. The level of statistical significance was set at $p < 0.05$.

Results and discussion

Effects of pioglitazone on insulin, leptin, glucose, triglyceride, triglyceride secretion rate, and post-heparin plasma LPL activity

Table 1 summarizes data on body weight, WAT weight, plasma insulin, TG, leptin, glucose, TGSR, and post-heparin plasma LPL activity of FVB and UCP–DTA mice. The body weight and WAT weight of UCP–DTA mice were significantly larger than wild-type control mice (FVB/N). The plasma level of insulin was 2.5-fold higher in UCP–DTA than in FVB mice, but plasma glucose levels were comparable between these animals, suggesting the development of insulin resistance in UCP–DTA mice. UCP–DTA mice had a plasma TG concentration that was 2.9-fold higher than that of FVB

Table 1
Characteristic features of FVB control, and UCP–DTA pre- and post-treated with pioglitazone (Pio.)

	FVB/N	UCP–DTA	
		Untreatment of Pio.	Post-treatment of Pio.
n	10	11	11
Initial body weight (g)	25.2 ± 0.7	29.6 ± 1.2[a]	29.2 ± 0.7
WAT weight (mg)	502.3 ± 51.2	682.9 ± 54.2[a]	519.6 ± 54.2
Plasma glucose (mg/dl)	165.0 ± 18.3	181.4 ± 9.9	164.6 ± 7.2
Plasma insulin (ng/ml)	3.0 ± 0.6	7.6 ± 1.4[a]	2.0 ± 0.3[b]
Plasma leptin (ng/ml)	3.4 ± 0.6	6.7 ± 0.9[a]	4.5 ± 1.0[b]
Plasma TG (mg/dl)	63.8 ± 11.7	182.4 ± 10.1[a]	143.6 ± 11.4[b]
TGSR (mg/min)	0.006 ± 0.002	0.04 ± 0.013[a]	0.032 ± 0.008
Post-heparine LPL (FFA release μmol/ml/min)	N/A	1.0 ± 0.2	1.6 ± 0.2[b]

Results are expressed as means ± SE. WAT weight in pre-treated UCP–DTA mice was used in other age-matched UCP–DTA mice ($n = 11$).
[a] $p < 0.05$ vs. FVB.
[b] $p < 0.05$ vs. UCP–DTA mice untreated with pioglitazone.

mice. UCP–DTA mice had a plasma leptin concentration that was 1.9-fold higher than that of FVB mice. UCP–DTA mice bred in our animal facility had hyperinsulinemia and hyperleptinemia due to insulin resistance and fatty obesity, which were consistent with Lowell's results [14]. Table 1 also shows the effects of pioglitazone on various measurements in UCP–DTA mice. Treatment with pioglitazone for 2 weeks did not affect food intake nor body weight gain in UCP–DTA mice and the final body weight was comparable between treated and untreated groups. Unlike untreated mice, WAT weight was not significantly increased in pioglitazone-treated UCP–DTA mice. Hyperinsulinemia was completely attenuated by treatment with pioglitazone in UCP–DTA mice without affecting the plasma glucose level, suggesting that insulin resistance was largely improved. Mantzoros et al. [15] reported severe leptin resistance in UCP–DTA mice. The hyperleptinemia in UCP–DTA mice was also improved by pioglitazone treatment, suggesting that pioglitazone promotes differentiation of adipocytes and reduces fat enriched adipocytes, which secrete more leptin [7]. Pioglitazone significantly decreased plasma TG concentration by 21% in UCP–DTA mice, but the TG concentration was still 2.2-fold higher than that of FVB mice. The triglyceride secretion rate (TGSR) in UCP–DTA mice was 6.7-fold larger than that in FVB

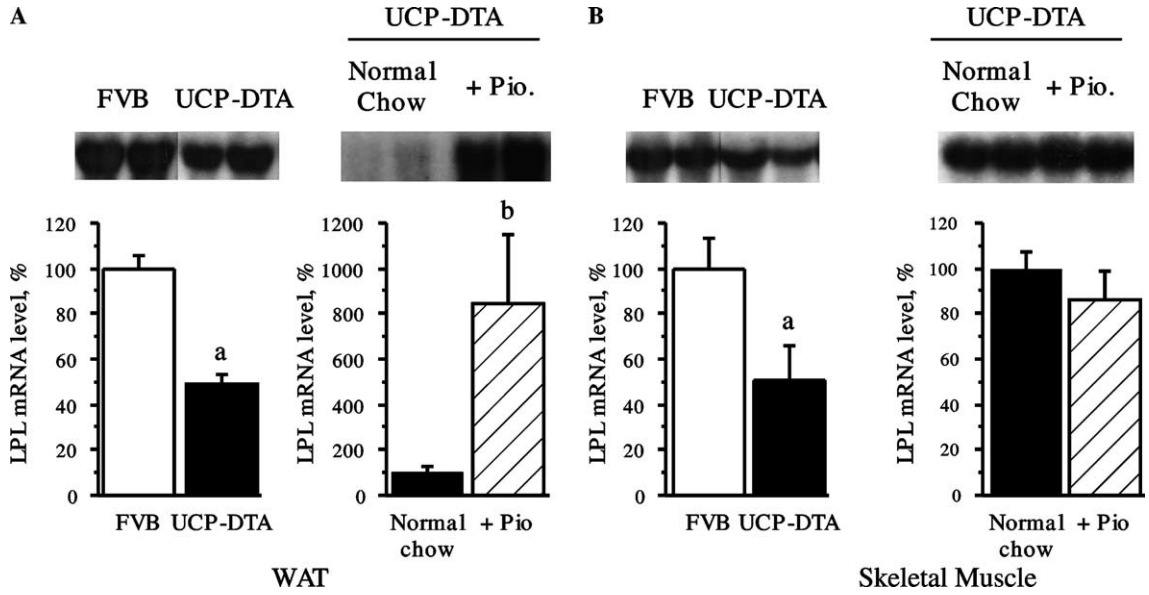

Fig. 1. Effect of 2-week treatment of pioglitazone (Pio.) on LPL mRNA in epididymal WAT (A) and lower limb skeletal muscle (B). Each value represents mean ± SE. The data for each band are shown as relative value to the LPL mRNA level of FVB/N control group or UCP–DTA mice treated without pioglitazone. FVB: FVB/N control mice ($n = 6$); UCP–DTA: UCP–DTA mice ($n = 4$); Normal Chow: UCP–DTA mice treated without pioglitazone ($n = 10$); +Pio.: UCP–DTA mice treated with pioglitazone ($n = 7$). [a] $p < 0.05$ vs. FVB/N control mice; [b] $p < 0.05$ vs. UCP–DTA mice untreated with pioglitazone (Normal Chow).

control mice. Pioglitazone did not decrease the high rate of TG production in UCP–DTA mice. We measured post-heparin plasma LPL activity in UCP–DTA mice treated or not with pioglitazone. LPL activity was slightly decreased in UCP–DTA mice. Treatment with pioglitazone increased LPL activity by 60%. These results suggest that the hypotriglyceridemic effect of pioglitazone in UCP–DTA mice is solely attributable to an improvement of TG catabolism via an increased LPL activity. The failure of pioglitazone to suppress the high rate of TG production may be the reason that the hypotriglyceridemic power of this agent is mild.

Effect of pioglitazone on the gene expression of LPL in epididymal WAT and lower limb skeletal muscles

Fig. 1 shows the expression of UCP2 in epididymal WAT and lower limb skeletal muscles of UCP–DTA and FVB control mice. LPL mRNA level in WAT of UCP–DTA mice was 50% that of FVB mice. LPL mRNA levels in skeletal muscles of UCP–DTA mice also decreased to 50% of FVB control mice. These findings indicate that decreased lipolysis by LPL as well as overproduction of TG contributes to the hypertriglyceridemia in UCP–DTA mice. Several studies have reported that insulin resistance decreases LPL content in representative LPL producing organs such as WAT [4,5] or skeletal muscle [5]. Our results suggest that LPL mRNA expression is decreased by insulin resistance and that there is no apparent organ specificity regarding insulin resistance and LPL synthesis. Pioglitazone remarkably increased LPL mRNA in WAT of UCP–DTA mice by 840% (Fig. 1). In contrast, LPL mRNA in skeletal muscle was not altered by the treatment with pioglitazone (Fig. 1). Increased LPL gene expression in WAT but unchanged LPL gene expression in skeletal muscle may explain the modest increase of post-heparin plasma LPL activity and the mild TG lowering effect of pioglitazone treatment. LPL plays an important role in the hydrolysis of TG-rich lipoprotein and delivery of fatty acid to organs. It is well known that increased fatty acid impairs glucose disposal, which is called lipotoxicity [21,22]. Thus, it is possible that an agent stimulating LPL biosynthesis in skeletal muscles worsens insulin resistance via lipotoxicity. In fact, mice overexpressing the human LPL gene in skeletal muscle develop severe insulin resistance [23–25]. In the present study, we observed that pioglitazone selectively increased LPL mRNA in WAT but not in skeletal muscles. No change of LPL production in skeletal muscles by pioglitazone would contribute to prevent the development of insulin resistance due to increased delivery of fatty acids to the muscles. Alternatively, the remarkable increase of LPL synthesis in WAT may in part explain the increased adiposity observed during long-term treatment with TZDs [26,27].

Effect of pioglitazone on gene expression of PPAR-γ and UCP2

We measured PPAR-γ mRNA in WAT of FVB and UCP–DTA mice treated or not with pioglitazone (Fig. 2). Expression of PPAR-γ mRNA was decreased by 60% in UCP–DTA mice as compared with FVB mice. The low gene expression of PPAR-γ in UCP–DTA mice may provide a molecular mechanism for the severe insulin resistance developed in these mice. We found that pioglitazone remarkably increased gene expression of PPAR-γ by 430% in UCP–DTA mice, suggesting that

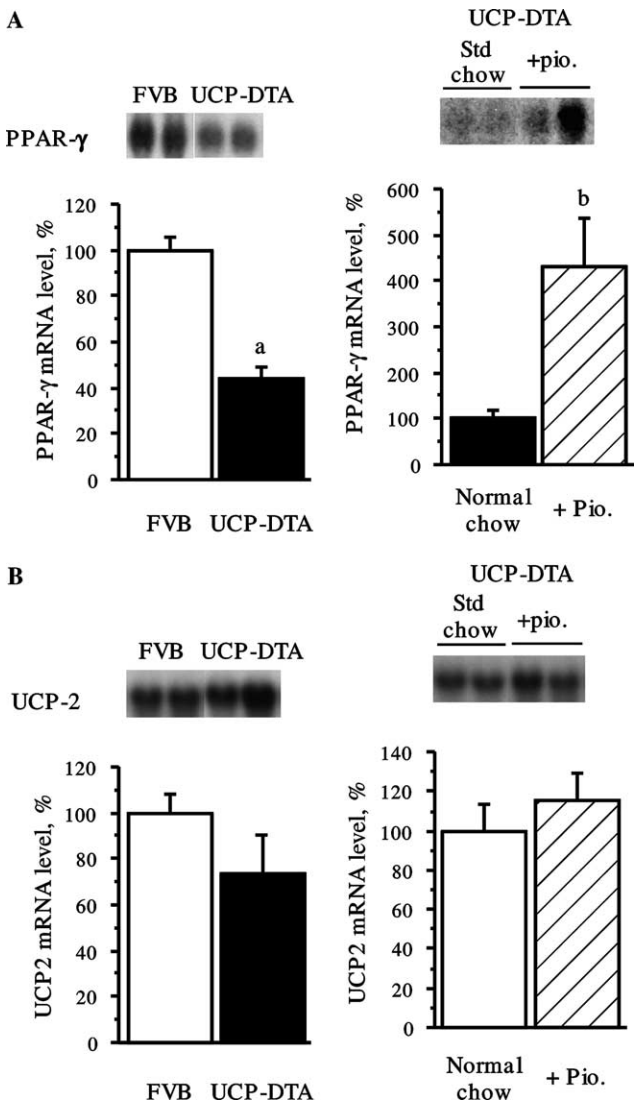

Fig. 2. Effect of 2-week treatment of pioglitazone (Pio.) on PPAR-γ (A) and UCP-2 (B) mRNA in epididymal WAT. Each value represents mean ± SE. The data for each band are shown as relative value to the mRNA level of FVB/N control group or UCP–DTA mice treated without pioglitazone. FVB: FVB/N control mice ($n = 6$); UCP–DTA: UCP–DTA mice ($n = 4$); Normal Chow: UCP–DTA mice treated without pioglitazone ($n = 10$); +Pio.: UCP–DTA mice treated with pioglitazone ($n = 7$). [a] $p < 0.05$ vs. FVB/N control mice; [b] $p < 0.05$ vs. UCP–DTA mice untreated with pioglitazone (Normal Chow).

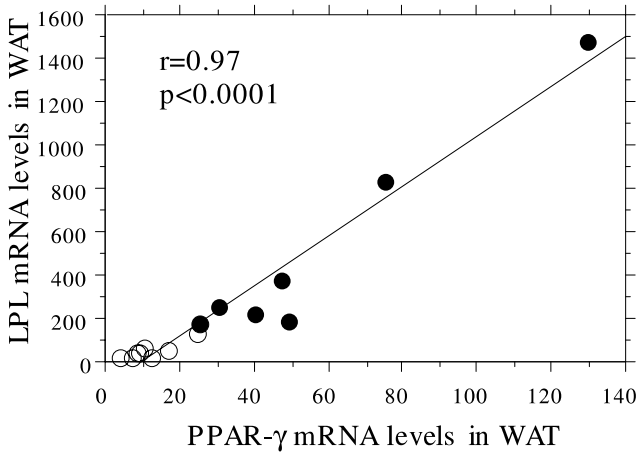

Fig. 3. Correlation of PPAR-γ mRNA expression with LPL mRNA expression in epididymal WAT of UCP–DTA treated without (open circle) and with (closed circle) pioglitazone. Each value was quantified using an image analyzer.

pioglitazone directly stimulates the transcription of PPAR-γ gene besides enhancing the action of PPAR-γ as its agonist. UCP2, which is a member of the UCP family, is expressed in ubiquitous tissues, including skeletal muscles [20,28]. Because UCP–DTA mice lacks the UCP-1 gene, we expected a change in UCP-2 gene expression to compensate the action of UCP-1. However, there was no significant difference in the level of UCP-2 mRNA between UCP–DTA and FVB mice. Pioglitazone did not affect UCP-2 mRNA in UCP–DTA mice. These results suggest that the change in insulin resistance in UCP–DTA mice was not associated with UCP-2 gene expression.

A transcriptionally active PPAR response element has been identified upstream of the LPL gene [6]. Recently, Lapsys et al. [29] reported that LPL mRNA in human skeletal muscles correlated with PPAR-γ expression in the same samples, suggesting that LPL biosynthesis is strongly governed by PPAR-γ. We found that LPL mRNA levels in WAT highly correlated with PPAR-γ mRNA levels in UCP–DTA mice treated or not with pioglitazone ($r = 0.97$, $p < 0.0001$) (Fig. 3). This provides an additional evidence that LPL gene is a target for the action of PPAR-γ.

In conclusion, LPL mRNA expression in WAT and skeletal muscles was decreased to similar extent in insulin resistant mice lacking BAT. However, pioglitazone, an enhancer of insulin action, selectively increased LPL mRNA expression in WAT but not in skeletal muscle. We speculate that stimulation of LPL gene expression by pioglitazone is attributable to the action of PPAR-γ, which abounds in WAT but not in skeletal muscles. The lack of effect of pioglitazone on gene expression of LPL in skeletal muscles would contribute to prevent the development of insulin resistance caused by excess fatty acid flux into the muscles.

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