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Thiazolidinediones Inhibit Lipoprotein Lipase Activity in Adipocytes*

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The thiazolidinediones troglitazone and BRL 49653 improve insulin sensitivity in humans and animals with insulin resistance. Adipose tissue lipoprotein lipase is an insulin-sensitive enzyme. We examined the effects of thiazolidinediones on lipoprotein lipase expression in adipocytes. When added to 3T3-F442A, 3T3-L1, and rat adipocytes in culture, troglitazone and BRL 49653 inhibited lipoprotein lipase activity. This inhibition was observed at concentrations as low as 0.1 μM and within 2 h after addition of the drug. Lipoprotein lipase activity was inhibited in differentiated adipocytes as well as the differentiating cells. Despite this decrease in enzyme activity, these drugs increased mRNA levels in undifferentiated 3T3-F442A and 3T3-L1 cells and had no effect on mRNA expression or synthesis of lipoprotein lipase in differentiated cells. Western blot analysis showed that these drugs did not affect the mass of the enzyme protein. Lipoprotein lipase activity in cultured Chinese hamster ovary cells was not inhibited by troglitazone. Glucose transport, biosynthesis of lipids from glucose or the biosynthesis of proteins were unaffected by thiazolidinediones in differentiated cells, whereas glucose transport and lipid biosynthesis were increased when these drugs were added during differentiation. These results show that troglitazone and BRL 49653 have a specific, post-translational inhibitory effect on lipoprotein lipase in adipocytes, yet they promote lipid accumulation and differentiation in preadipocytes.

Adipocytes are derived from a stem cell of mesodermal origin, and adipocyte differentiation is characterized by a coordinate increase in the expression of a number of specific genes. Lipoprotein lipase (LPL)[1]-mediated triglyceride hydrolysis is central to adipocyte lipid accumulation in humans and rats (1–3) and is expressed early in adipocyte differentiation.

Peroxisome proliferator-activated receptor γ (PPARγ), a member of the nuclear receptor superfamily of ligand-activated transcription factors, is expressed during differentiation of several preadipocyte cell lines (4), and the forced expression of PPARγ in fibroblasts resulted in differentiation of these cells into adipocytes (5). PPARs regulate the expression of genes by binding to the PPAR response elements, which have been identified in the regulatory regions of several genes encoding enzymes that modulate the metabolism of lipids (6, 7).

Thiazolidinediones are antidiabetic agents that lower blood glucose by increasing the insulin sensitivity of target tissues in human subjects and animal models of non-insulin-dependent diabetes (NIDDM) (8). These drugs also decrease plasma insulin and triglycerides in insulin-resistant animal models (9). Thiazolidinediones were also found to activate PPARγ in preadipocytes and promote differentiation into adipocytes (10). Pioglitazone, one of the thiazolidinedione compounds, increased the rate of lipogenesis and glucose transport in 3T3-L1 cells and increased the expression of mRNA for Glut4, LPL, and glucose-6-phosphate dehydrogenase in the presence of insulin (11). In another study, a similar enhancement of adipocyte differentiation by pioglitazone was observed in 3T3-F442A cells (12). Tafuri (13) has recently reported that troglitazone enhanced the differentiation, basal glucose transport, and Glut1 level in 3T3-L1 cells, and Schoonjans *et al.* (14) demonstrated an increase in LPL mRNA levels in undifferentiated 3T3-L1 cells following treatment with BRL 49653. In none of these studies was the activity of LPL determined.

In the present study, we investigated the effect of troglitazone and BRL 49653 on LPL expression and enzyme activity in 3T3-F442A, 3T3-L1 cells, and rat adipocytes. As reported by other investigators, these drugs stimulated the expression of LPL mRNA in 3T3- preadipocytes (14). However, they inhibited LPL activity in differentiated adipocytes and isolated rat adipocytes suggesting post-transcriptional inhibition of LPL expression. When added to 3T3-F442A preadipocytes, despite decreased LPL activity, thiazolidinediones increased the rate of glucose transport, lipid biosynthesis, and lipid accumulation.

EXPERIMENTAL PROCEDURES

Materials—3T3-F442A and 3T3-L1 cells were obtained from Dr. Howard Green (Massachusetts Institute of Technology, Cambridge). Media and other tissue culture supplies were from Life Technologies, Inc. Human recombinant insulin (Novolin) was obtained from Nordisk Pharmaceuticals (Princeton, NJ). [U-^{14}C]Glucose, 2-deoxy-[^{3}H]glucose, [^{35}S]methionine, and [^{3}H]triolein were from NEN Life Science Products. Bovine serum albumin, triolein, lecithin, clofibrate, prostaglandin D_2, docosahexaenoic acid, 2-deoxyglucose, and peroxidase-conjugated anti-rabbit chicken IgG were purchased from Sigma. Matrigel was obtained from Becton Dickinson. Troglitazone was a gift from Parke-Davis. BRL 49653 was obtained from Smith Kline Beecham (United Kingdom). Polyclonal antibodies against bovine LPL raised in rabbit were from Dr. Ira Goldberg (Columbia University, New York).

Cell Culture—3T3-F442A cells were maintained in 75-cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and a mixture of penicillin and streptomycin. For experiments, they were subcultured in 12-well dishes. Confluent cultures were allowed to differentiate by adding DMEM containing 10% fetal bovine serum and 100 nM insulin. The cells differentiated well in 3–5 days after switching to the differentiation medium. 3T3-L1 cells were differentiated using isobutylmethylxanthine (IBMX), dexamethasone,

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[1] The abbreviations used are: LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; NIDDM, non-insulin-dependent diabetes mellitus; IBMX, isobutylmethylxanthine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum.

and insulin as described by Clancy and Czech (15). Troglitazone and BRL 49653 were dissolved in dimethyl sulfoxide and added in the concentrations indicated. The stock solutions were made in such a way that no more than 2 μl were added to 1 ml of culture medium. The control cells received 2 μl of dimethyl sulfoxide per ml of medium. The cell number was determined in a Coulter counter (Coulter Electronics, Hialeah, FL). In some experiments, cells were cultured in lipid-depleted FBS. This was obtained by extraction of FBS twice with heptane and then ultracentrifugation after adjusting the density to 1.2 g/ml with KBr. The floating lipid layer was discarded, and the infranatant was collected and dialyzed extensively with phosphate-buffered saline.

Isolation and Culture of Rat Adipocytes—Male Sprague-Dawley rats weighing between 150 and 250 g were sacrificed, and the epididymal adipose tissue was removed immediately. Adipocytes were isolated from the adipose tissue by collagenase digestion (16). The cells were washed three times and cultured in Matrigel-coated 12-well culture dishes. Matrigel was thawed on ice, and 150 μl of the liquid was applied to each well in the plate kept on ice. 150 μl of the adipocyte suspension (1:1 ratio of packed cells to medium) were added to each well on the liquid matrix and incubated for 30 min at 37 °C. During this time the Matrigel gelled around the cells and anchored them to the dish. After this incubation 2 ml of DMEM containing 10% FBS was added to each well and incubated for 24 h. The medium was then replaced with fresh medium, and troglitazone or BRL 49653 were added to give a final concentration of 1 μM. After 24 h the medium was aspirated, and the cells were incubated in heparin-containing medium for 1 h to release the LPL.

Glucose Transport—Before the glucose transport assay the cells were incubated with 1 ml of serum-free DMEM for 4 h. The cells were then washed twice with Krebs-Ringer phosphate buffer and incubated in the same buffer for 30 min. 2-Deoxy[^{3}H]glucose was added to give a final concentration of 0.1 mM (1 μCi/100 nmol) and incubated for 10 min. The medium was aspirated, and the cells were washed three times and dissolved in 0.5 ml of 0.2 N NaOH. Radioactivity in this lysate was determined by scintillation counting. Protein content in the lysate was determined by Bio-Rad reagent. Non-carrier-mediated glucose transport was determined in the presence of 10 μM cytochalasin (15).

LPL Assay—LPL was measured in the medium after release with heparin, as described previously (17). The culture medium was removed from the dishes, and 1 ml of fresh medium containing heparin (10 units/ml) was added and incubated for 60 min at room temperature. It was found that more than 90% of the total extractable LPL activity in these cells was released by heparin under these conditions. LPL catalytic activity was measured using an emulsified [^{3}H]triolein substrate as described previously (18). After incubation of 100 μl of sample with 100 μl of substrate for 60 min, liberated ^{3}H-fatty acids were separated from the reaction mixture using the method of Belfrage and Vaughn (19). The LPL activity is expressed as nanomoles of fatty acids released in 60 min.

Incorporation of [^{14}C]Glucose and [^{14}C]Acetate into Lipids—To study the biosynthesis of total lipids from glucose, the cells were incubated with [U-^{14}C]glucose or [^{14}C]acetate for the indicated times and washed five times with phosphate-buffered saline. Total lipids from the monolayers were extracted using 2 ml of hexane:isopropyl alcohol (3:2) mixture and evaporated to dryness, and then the radioactivity was determined.

Incorporation of [^{35}S]Methionine into Protein—The cells were incubated with [^{35}S]methionine for 2 h. The monolayers were washed five times with phosphate-buffered saline and dissolved in 1 ml of 0.2 N NaOH in a microcentrifuge tube. These cell extracts were treated with trichloroacetic acid to give a final concentration of 15% for 2 h on ice. The tubes were centrifuged, and the pellets were washed three times with 10% trichloroacetic acid. The pellets were finally dissolved in 0.2 N NaOH, and the radioactivity was determined.

RNA Extraction and Northern Blot Analysis—RNA was isolated from the cells using guanidine thiocyanate as described by Chomczynski and Sacchi (20). Equal amounts of RNA were electrophoresed on a 1% agarose-formaldehyde gel, transferred to nylon membrane (Nylon 1, Life Technologies, Inc.), and hybridized with ^{32}P-labeled cDNA probes for human LPL. The blot was probed simultaneously for glyceraldehyde phosphate dehydrogenase to control the amount of RNA loaded in the gel.

[^{35}S]Methionine Labeling and Immunoprecipitation of LPL—After treating the cells with troglitazone the culture medium was removed and replaced with methionine-free medium. They were pulsed with 50 μCi of ^{35}S-labeled methionine for 30 min. At the end of this period the medium was aspirated, and the cells were lysed in a buffer containing detergents. Quantitative immunoprecipitation with anti-LPL antibody

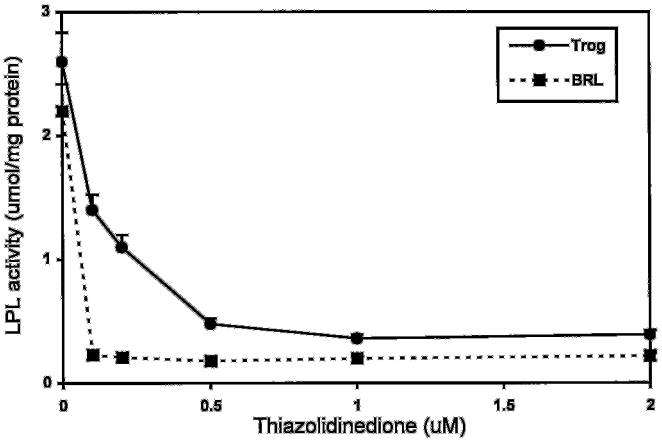


FIG. 1. **Effect of troglitazone and BRL on LPL activity in differentiated 3T3-F442A adipocytes.** Differentiated 3T3-F442A adipocytes were treated with the indicated concentrations of troglitazone (*Trog*) or BRL for 16 h. The cells were then washed and the heparin-releasable LPL activity was determined as described under "Experimental Procedures." Each value represents mean from analyses in triplicate dishes, and the *error bars* indicate S.D.

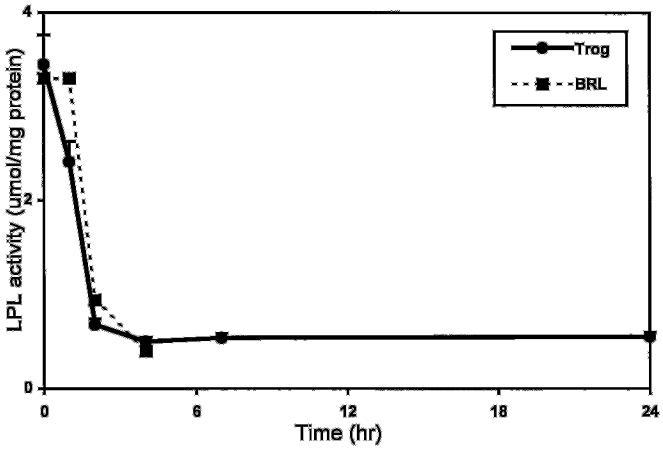


FIG. 2. **Time course of inhibition of LPL activity by troglitazone and BRL 49653 in differentiated 3T3-F442A adipocytes.** Differentiated adipocytes were treated with 1.0 μM troglitazone (*Trog*) or BRL for the indicated periods, and the heparin-releasable LPL activity was determined as described under "Experimental Procedures." Each value represents mean from triplicate dishes, and the *error bars* indicate S.D.

TABLE I
Effect of troglitazone and BRL 49653 on LPL activity in differentiated 3T3-L1 adipocytes

3T3-L1 cells were differentiated as described under "Experimental Procedures." The differentiated cells were treated with 1 μM troglitazone or BRL 49653 for 5 h, and the heparin-releasable LPL activity was determined. The values are mean and S.D. from triplicate wells for each treatment.

Additions	LPL activity
	nmol/mg cell protein
None	620 ± 12
Troglitazone	150 ± 10
BRL	110 ± 30

was carried out using samples containing an equal amount of trichloroacetic acid-precipitable counts, followed by electrophoresis on 10% SDS-polyacrylamide gel electrophoresis and radioautography (21).

Western Blot Analysis—The cells from each well from a 12-well plate were lysed in 1.0 ml of a buffer containing Triton X-100 and phenylmethylsulfonyl fluoride. The lysates were centrifuged in a microcentrifuge, and 5 μl of the clear samples were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose

membrane. LPL was detected using polyclonal antibodies raised against bovine LPL in rabbit and peroxidase-conjugated second antibody. The bands were visualized using chemiluminescent reagents (Amersham Pharmacia Biotech).

Statistical Analysis—All experiments were carried out at least two times in triplicate wells and processed individually. Data are expressed as the mean ± S.D. Student's *t* test was used for statistical analysis.

RESULTS

To examine the effects of troglitazone and BRL 49563 on LPL activity, 3T3-F442A cells were induced to differentiate, as described under "Experimental Procedures," and the drugs were added to the differentiated cells. The data presented in Fig. 1 show that the addition of troglitazone inhibited LPL activity in a dose-dependent manner. At a troglitazone concentration of 0.1 μM, the inhibition was 46%, and at 1.0 μM the inhibition was maximal at 86%. BRL 49653 inhibited 90% of LPL activity at 0.1 μM. Similar experiments were done to determine the time course of LPL inhibition by these drugs. By using these drugs at a concentration of 1.0 μM, the inhibition of LPL activity can be observed 1 h after addition of troglitazone and 2 h after addition of BRL 49563, with a maximal inhibition after 4 h (Fig. 2). LPL activity was also inhibited by these drugs when added to cultured rat adipocytes at 1 μM concentration for 16 h (control, 44.6 ± 5.1: troglitazone, 25.1 ± 2.8: 23.7 ± 2.3; all values are expressed as nmol/10^6 cells ± S.D.). The inhibition of LPL activity was also observed in 3T3-L1 cells differentiated by treatment with IBMX, dexamethasone, and insulin (Table I). There was no direct inhibition of LPL triglyceride hydrolysis by troglitazone when the drug was added directly to an LPL source *in vitro* (data not shown). The inhibition of LPL by troglitazone was partially reversible when the drug was withdrawn. As shown in Fig. 3, LPL activity was inhibited by about 75% when the differentiated adipocytes were treated with the drug for 16 h. After this treatment, when the cells were washed and incubated without the drug, the LPL activity recovered to about 60% of the control value within 4 h.

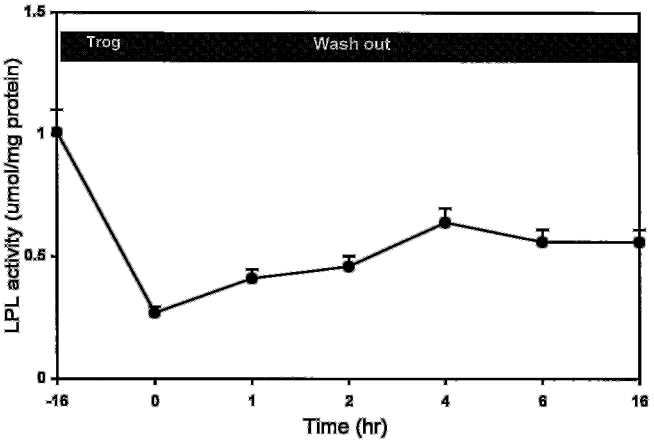


FIG. 3. **Effect of removal of troglitazone after treatment on LPL activity.** Differentiated 3T3-F442A adipocytes were treated with 1.0 μM troglitazone (*Trog*) for 16 h. The cells were then washed and incubated without troglitazone for the indicated periods before assaying heparin-releasable LPL activity. The values are means and S.D. from triplicate dishes.

When cultured Chinese hamster ovary cells, which express LPL, were treated with troglitazone, no inhibition of LPL activity was observed (control, 94 nmol/mg protein; troglitazone-treated cells (1 μM, 18 h), 116 nmol/mg cell protein). The inhibitory effect of troglitazone on LPL activity appears to be relatively specific to adipocytes.

The above experiments involved differentiated adipocytes. We next examined the effect of adding troglitazone and BRL 49653 to 3T3-F442A cells during differentiation. As shown in Table II, LPL activity increased about 4-fold during differentiation with insulin alone. When the cells were treated with thiazolidinedione for 4 days during the differentiation process, LPL activity was decreased both in the presence and absence of insulin. During this time, despite the decreased LPL activity, the cells had accumulated lipid droplets and differentiated into adipocytes, and the cellular triglyceride content had increased (Table II). These data show that troglitazone and BRL 49653 inhibited LPL activity in differentiating as well as fully differentiated 3T3-F442A adipocytes.

In order to understand how the cells were able to accumulate lipids in the presence of a thiazolidinedione-mediated inhibition of LPL activity, we studied the uptake of glucose and the biosynthesis of lipids from [^{14}C]glucose and [^{14}C]acetate. The data in Table II show that glucose transport increased about 12-fold and glucose incorporation into lipids by 6-fold in troglitazone-treated cells. The presence of insulin along with troglitazone increased the glucose uptake and lipid biosynthesis further. Troglitazone and BRL 49653 also stimulated the synthesis of lipids from [^{14}C]acetate about 12- and 20-fold, respectively.

Although troglitazone inhibited LPL activity, it did not affect other metabolic pathways such as glucose transport, lipid biosynthesis, or protein synthesis in differentiated 3T3-F442A adipocytes as shown in Table III. This suggests that the inhibition of LPL activity by troglitazone is a specific effect and not

TABLE III

Effect of troglitazone on glucose transport, [^{14}C]glucose incorporation into total lipids, and [^{35}S]methionine incorporation into proteins in differentiated 3T3-F442A adipocytes

The differentiated 3T3-F442A adipocytes (4 days with 100 nM insulin) were incubated with 1.0 μM troglitazone for 4 h. They were then pulsed with 0.5 μCi of 2-deoxy-[^{3}H]glucose for 10 min, or 0.1 μCi of [U-^{14}C]glucose (265 mCi/mmol) for 2 h, or with 50 μCi of [^{35}S]methionine (1400 Ci/mmol) for 2 h. The methods for analyzing the incorporation of these tracers into lipids and proteins are described under "Experimental Procedures." The values are means ± S.D. from analyses of three dishes.

Additions	Glucose transport	[^{14}C]Glucose lipids	[^{35}S]Methionine proteins
	nmol/mg protein	*dpm/mg protein*	
None	3.37 ± 0.18	38,680 ± 450	10,260 ± 300
Troglitazone	3.99 ± 0.07	35,000 ± 2050	11,050 ± 250

TABLE II

Effect of troglitazone on LPL activity, 2-deoxyglucose transport, cellular triglyceride content, and lipid biosynthesis in 3T3-F442A cells

The cells were cultured in 12-well dishes, and when the monolayers were confluent they were treated with insulin (100 nM), troglitazone (Trog) (1.0 μM), BRL 49653 (1.0 μM) for 4 days. At the end of this period more than 70% of the cells were differentiated into adipocytes. LPL activity and 2-deoxyglucose transport were assayed as described under "Experimental Procedures." All values are per 10^6 cells.

Additions	LPL activity	Glucose transport	Triglycerides	Glucose → lipids	Acetate → lipids
	nmol	*nmol*	*μg*	*nmol*	*dpm × 10^{-3}*
None	123 ± 8	0.23 ± 0.04	3.3 ± 0.2	8.0 ± 0.1	14 ± 2
Insulin	475 ± 30	3.7 ± 0.4	5.5 ± 0.4	42 ± 1.9	123 ± 3
Troglitazone	48 ± 5	2.7 ± 0.2	9.5 ± 0.6	49 ± 2.8	163 ± 23
Insulin + Trog	84 ± 6	15.4 ± 0.8	10.5 ± 0.5	106 ± 12	
BRL 49653	30 ± 3		13.2 ± 1.1		270 ± 26

TABLE IV

Effect of troglitazone and BRL 49563 on cell number, cell protein, and protein synthesis in differentiating 3T3-F442A cells

Confluent monolayers of 3T3-F442A cells were treated with 1 μM troglitazone, BRL 49653, or 100 nM insulin for 4 days. At the end of this period the cell number was determined after releasing them by trypsinization. For protein measurement the cells were lysed in 0.2 M NaOH. To measure the rate of protein synthesis, the monolayers were pulsed with 1 μCi of [^{35}S]methionine for 1 h, and the amount of trichloroacetic acid-precipitable radioactivity in the cells was determined. All values are means ± SD from triplicate analysis.

Additions	Cell number	Cell protein	Protein synthesis
	$\times 10^{-6}$/well	μg/well	dpm $\times 10^{-3}/10^{6}$ cells
None	0.21 ± 0.02	49 ± 1	74 ± 16
Troglitazone	0.27 ± 0.04[a]	110 ± 6[a]	198 ± 56[a]
BRL	0.19 ± 0.02	116 ± 7[a]	280 ± 27[a]
Insulin	0.30 ± 0.05[a]	118 ± 5[a]	127 ± 31[a]

[a] $p < 0.05$ *versus* no additions.

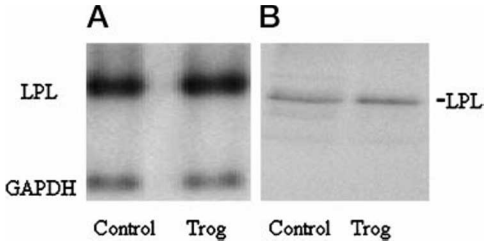


FIG. 4. **Effect of troglitazone and on the expression of LPL mRNA and the synthesis of LPL protein from ^{35}S-labeled methionine.** *A,* Northern blot analysis. The differentiated 3T3-F442A cells were treated with 1.0 μM troglitazone (*Trog*) for 24 h. The experimental details are described under "Experimental Procedures." Each lane had 15 μg of total RNA. *B*, synthesis of LPL from [^{35}S]methionine. Differentiated adipocytes were treated with 1 μM troglitazone for 4 h and then pulsed with 50 μCi of [^{35}S]methionine for 30 min. *GAPDH,* glyceraldehyde phosphate dehydrogenase. Experimental details are as described under "Experimental Procedures."

due to drug-mediated cell toxicity.

The addition of troglitazone and BRL 49653 also increased the cellular protein content by 3-fold, but the cell number varied only to a small but significant extent. The rate of protein synthesis was studied, and the results show that both these drugs increased the incorporation of [^{35}S]methionine into proteins 3–4-fold (Table IV).

Experiments were carried out to determine at which level of gene expression the inhibition of LPL activity occurred. Bu using differentiated adipocytes, Northern blot analysis of the RNA isolated from control and troglitazone-treated cells showed that there was no change in the expression of LPL mRNA (Fig. 4*A*). Since the regulation of LPL is known to occur at the level of translation, we also studied the rate of incorporation of [^{35}S]methionine into immunoprecipitable LPL protein. The results, presented in Fig. 4*B*, show that troglitazone did not inhibit the synthesis of LPL protein. Western blots of the cell extracts also demonstrated no change in LPL protein in the total as well as the heparin-releasable fractions despite the inhibition of enzyme activity by more than 65% (Fig. 5).

Schoonjans *et al.* (14) have reported that BRL 49653 increased the expression of LPL mRNA in 3T3-L1 preadipocytes when they were cultured in medium containing lipid-depleted FBS. Such culture conditions permit growth of these cells for longer periods without undergoing differentiation (4). To examine the effects of thiazolidinediones on LPL mRNA levels in undifferentiated cells, we added troglitazone and BRL 49653 to 3T3-L1 preadipocytes, in the absence of differentiation agents, and observed an increase in LPL mRNA expression (Fig. 6). This effect was more pronounced when the cells were cultured in lipid-depleted FBS. However, even though 3T3-L1 preadipocytes demonstrated increased LPL mRNA following treatment with thiazolidinediones, these cells expressed low levels of LPL activity, which was no different between control and thiazo-

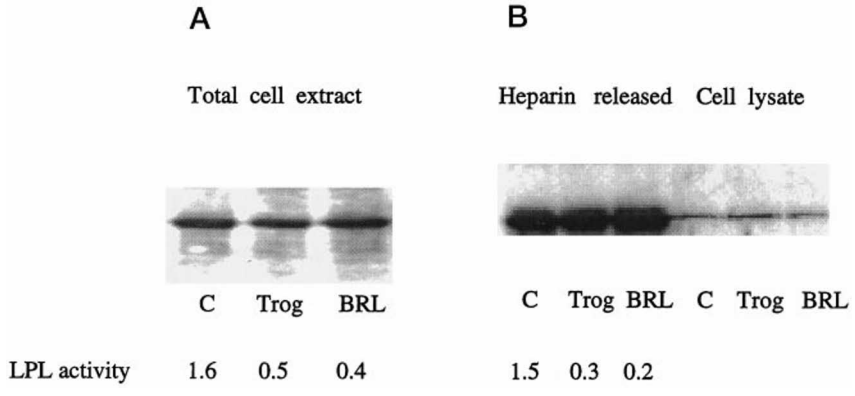
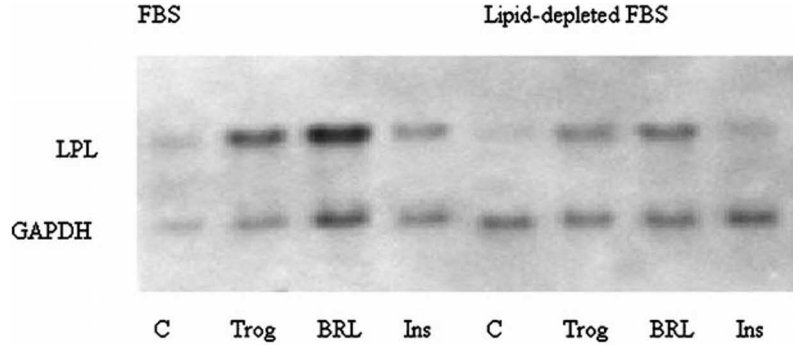


FIG. 5. **Effect of troglitazone and BRL 49563 on the cellular LPL protein content in differentiated 3T3-F442A adipocytes.** *A,* the cells were treated with 1 μM troglitazone (*Trog*) or BRL 49653 for 16 h. Western blot analysis was carried out as described under "Experimental Procedures," using equal amounts of the cell lysate. LPL assays were carried out in total cell extracts from another set of plates treated under identical conditions. *B*, the experimental conditions were same as described for *A*. Electrophoresis was carried out using equal amounts of the heparin-releasable fraction and the extractable cell material after heparin release. The LPL activities shown are μmol/mg cell protein. *C*, control.

FIG. 6. **Expression of LPL mRNA in 3T3-L1 preadipocytes.** 3T3-L1 cells were cultured in DMEM containing calf serum until they were about 80% confluent. They were then switched to media containing either 10% FBS or lipid-depleted FBS and cultured for 4 days in the presence of 1 μM troglitazone (*Trog*), BRL 49653 (*BRL*), or 100 nM insulin (*Ins*). RNA was isolated and subjected to Northern blot analysis (10 μg/lane). The blots were hybridized with ^{32}P-labeled cDNA probes for LPL and glyceraldehyde phosphate dehydrogenase (*GAPDH*). *C*, control.



lidinedione-treated cells and not increased with insulin (Table V). These cells expressed high LPL activity only when they were differentiated with IBMX, dexamethasone, and insulin.

Similar experiments were carried out with 3T3-F442A cells. When 3T3-F442A cells were cultured in lipid-depleted FBS, both troglitazone and BRL stimulated LPL mRNA levels (Fig. 7), but LPL activity was low under these conditions, and treatment with thiazolidinediones did not affect LPL activity (Table V). In the presence of FBS, 3T3-F442A cells demonstrated some spontaneous differentiation, manifested by an increase in LPL and LPL inhibition by thiazolidinediones (Table V).

In order to determine if the inhibition of LPL by troglitazone was related to PPARγ activation, the effect of other compounds which are known to be PPARγ activators was tested. As shown in Table VI, treatment of 3T3-F442A adipocytes with 1 μM troglitazone and BRL 49653 inhibited LPL activity by about 90%. The other compounds, clofibrate, prostaglandin D_2, and docosahexaenoic acid at the relatively high concentrations that are typically used to stimulate PPARγ, also inhibited LPL activity by 77, 63, and 62%, respectively. At a concentration of 1 μM, these compounds inhibited LPL less.

DISCUSSION

Thiazolidinediones are drugs that have been shown to have multiple effects in cultured cells. When added to preadipocytes in culture, thiazolidinediones stimulated lipid accumulation and stimulated the expression of the enzymes of adipocyte differentiation (11, 12, 22, 23). This promotion of differentiation has been demonstrated to be stimulated by agents that bind to PPARγ (10), including the thiazolidinediones. Among the genes that are activated during adipocyte differentiation are aP2, adipsin, LPL, leptin, and Glut-4 (24). Because of the important role of LPL in adipocyte lipid accumulation, one would expect that any stimulation of adipocyte differentiation would also result in the activation of LPL activity. Indeed, Kleitzien *et al.* (11) showed that pioglitazone increased LPL mRNA expression in 3T3-L1 cells 3-fold when added in the presence of insulin during differentiation. However, no change was observed when the drug was added in the absence of insulin. Szalkowski *et al.* (22) found that another thiazolidinedione, CP-86,325, did not affect LPL expression in differentiated 3T3-L1 cells. Schoonjans *et al.* (14) found that BRL 49653 stimulated the expression LPL mRNA in undifferentiated 3T3-L1 cells. However, none of these *in vitro* studies examined LPL activity.

The data presented in this paper show that troglitazone and BRL 49653 inhibited LPL activity in 3T3-F442A adipocytes, 3T3-L1 adipocytes, and rat adipocytes. This inhibitory effect was observed in the cells that were in the process of differentiation as well as in fully differentiated cells. Although LPL activity was inhibited by thiazolidinediones, LPL mRNA was either unchanged, in the case of differentiated adipocytes, or increased, in the case of preadipocytes. LPL translation and the immunoreactive mass of LPL as measured by Western blotting were unaffected. Thus, thiazolidinediones appear to cause a potent inhibition of LPL post-translational processing resulting in the synthesis of LPL with a lower specific activity. Previous studies have demonstrated that the regulation of LPL is complex, and regulation may occur at the level of transcription, translation, or post-translational processing (21, 25–28). In addition, the insulin-stimulated increase in LPL activity in 3T3-L1 cells is primarily due to post-translational mechanisms (29). These data would suggest that thiazolidinediones inhibit the formation of active LPL, which could be due to a prevention of glucose trimming of the *N*-linked oligosaccharide or the interruption of transfer from the endoplasmic reticulum to the Golgi (30, 31). Such a

TABLE V

Effect of troglitazone and BRL 49653 added during differentiation in 3T3-L1 and 3T3-F442A cells cultured in FBS and lipid-depleted FBS on LPL activity

3T3-F442A and 3T3-L1 cells were cultured in DMEM containing FBS until they were about 80% confluent. The medium was then changed to either FBS or lipid-depleted FBS containing DMEM in the presence of 1 μM troglitazone or BRL 49653 or 100 nM insulin as indicated, and cultured for 4 days. Heparin-releasable LPL activity was determined as described under "Experimental Procedures." The values are means ± S.D. from triplicate analysis.

	LPL activity, nmol/10^6 cells	
	3T3-F442A	3T3-L1
FBS		
Control	495 ± 100	185 ± 21
Troglitazone	175 ± 30[a]	195 ± 13
BRL	120 ± 10[a]	195 ± 20
Insulin	1360 ± 50[a]	175 ± 14
Lipid-depleted FBS		
Control	65 ± 8	70 ± 13
Troglitazone	85 ± 16	70 ± 14
BRL	65 ± 14	75 ± 13
Insulin	360 ± 38[a]	78 ± 3

[a] $p < 0.05$ versus control.

TABLE VI

Inhibition of LPL activity in differentiated 3T3-F442A adipocytes by activators of PPARγ

Differentiated 3T3-F442A adipocytes were treated with the indicated compounds for 4 h, and the heparin releasable LPL activity was determined. The results are expressed as percent of the remaining LPL activity compared with the untreated control cells.

Additions	Concentration	LPL activity
		% control
None		100
Troglitazone	1 μM	9 ± 2
BRL 49653	1 μM	8 ± 3
Clofibrate	1 μM	77 ± 8
	10 mM	23 ± 4
Prostaglandin D2	1 μM	46 ± 7
	10 μM	37 ± 5
Docosahexaenoic acid	1 μM	73 ± 9
	50 μM	38 ± 6

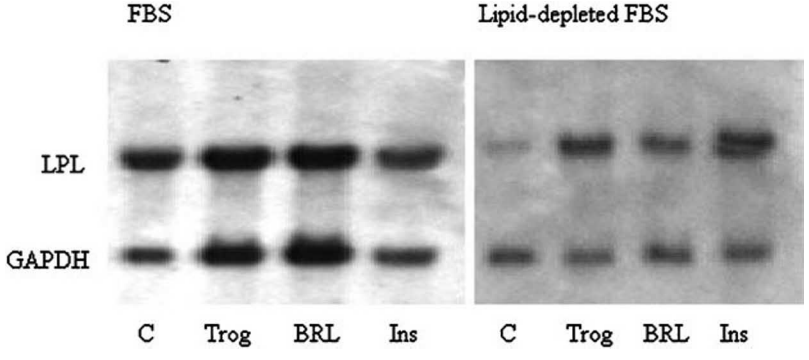


FIG. 7. **Expression of LPL mRNA in 3T3-F442A preadipocytes.** The experimental details are exactly same as those used for 3T3-L1 cells described in the legend for Fig. 6.

mechanism for LPL post-translational regulation occurs with fasting (30). However, previous studies have found that the inhibition of LPL post-translational processing was also associated with decreased secretion (30–32). Our studies describe the secretion of inactive LPL, suggesting that thiazolidinediones are able to uncouple LPL processing from LPL secretion.

Although LPL activity in adipocytes was inhibited, troglitazone and BRL 49653 stimulated glucose transport and lipid accumulation when added during differentiation. These data are similar to those reported by others (12, 13) and suggest that the increase in lipid accumulation is due to the activation of *de novo* lipid biosynthesis, possibly involving enzymes in fatty acid biosynthesis. Mammalian adipocytes are generally dependent on LPL-mediated triglyceride hydrolysis for lipid accumulation and rely little on *de novo* lipid biosynthesis (2, 3, 31). Thus, these data are unusual since 3T3 preadipocytes treated with thiazolidinediones are able to accumulate lipid with very low LPL activity.

Thiazolidinediones are potent activators of PPARγ and are known to regulate the expression of adipocyte-specific genes through PPARγ (7, 10). In these previous studies, the time required to observe changes in response to thiazolidinediones ranged from 1 to 4 days. We also found an increase in LPL mRNA after 4 days of treatment with troglitazone. However, we observed an inhibition of LPL activity within 1 to 2 h after the addition of troglitazone or BRL 49653. Parallel experiments with other known PPARγ-stimulating agents indicate that they also inhibit LPL activity, although to a lesser extent. Therefore it appears that the inhibition of LPL activity may be an early event following PPARγ activation. Rapid activation of PPARγ may transcriptionally activate some other factor that inhibits the processing of the LPL molecule from the inactive nascent form to the active form.

It is also possible that troglitazone and BRL 49653 have effects that are not mediated through PPARγ activation. For example, a number of studies have described effects of troglitazone outside of adipocytes. Fulgencio *et al.* (33) have shown that troglitazone inhibited fatty acid oxidation and gluconeogenesis in isolated rat hepatocytes, where PPARγ expression was absent or very low (34, 35). Troglitazone was also found to decrease the oxidation of low density lipoprotein and high density lipoprotein *in vitro* by copper ions and endothelial cells (36). In another study Law *et al.* (37) have reported that troglitazone inhibited the growth of vascular smooth muscle cells. All these studies raise the possibility that troglitazone may have various metabolic effects on different cells with or without any involvement of PPARγ activation, and the inhibitory effect of troglitazone and BRL 49653 on LPL activity may be independent of PPARγ activation.

Whether or not the *in vitro* inhibition of LPL in adipocytes corresponds to the clinical role of thiazolidinediones is unclear. In animal models of NIDDM, troglitazone lowered plasma triglycerides (8). Whether this was due to a decrease in very low density lipoprotein production or an increase in LPL-mediated removal is not clear, although Schoonjans *et al.* (14) and Lefebvre *et al.* (38) have demonstrated an increase in adipose tissue LPL activity in rats given BRL 49653. However, it is not known whether the increase in adipose tissue LPL activity in thiazolidinedione-treated rats was due to a direct effect of the drug or due to the improved insulin sensitivity. Previous studies have shown that any treatment that resulted in an improvement in glycemic control in patients with NIDDM or an improvement in insulin action would result in both a decrease in very low density lipoprotein output and an increase in LPL activity (39–42). Thus, the difference between *in vivo* and *in vitro* effects of thiazolidinediones may be due to the improvement in insulin action *in vivo*, with an increase in LPL secondary to the reduction in insulin resistance.

Burant *et al.* (43) have recently reported that troglitazone treatment decreased plasma glucose, insulin, triglycerides, and fatty acids in mice in which both white and brown adipose tissue were ablated by targeted expression of diphtheria toxin A chain directed by adipose-specific aP2 promoter. They suggested that the antidiabetic and hypolipidemic effects of troglitazone are independent of adipose tissue. Thus, it is possible that thiazolidinediones have direct effects in adipocytes which, in the whole animal, are overcome by the extra adipose tissue effects, which yield an improvement in whole animal insulin action.

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REFERENCES

1. Hollenberg, C. H. (1966) *J. Clin. Invest.* **45,** 205–216
2. Wilson, J. P., Gutman, R., and Galton, D. J. (1973) *Metabolism* **22,** 913–921
3. Shrago, E., Spennetta, T., and Gordon, E. (1969) *J. Biol. Chem.* **244,** 2761–2766
4. Chawla, A., and Lazar, M. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1786–1790
5. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156
6. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahli, W. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 2160–2164
7. Harris, P. K. W., and Kletzien, R. F. (1993) *Mol. Pharmacol.* **45,** 439–445
8. Saltiel, A. R., and Olefsky, J. M. (1996) *Diabetes* **45,** 1661–1169
9. Stevenson, R. W., Hutson, N. J., Krupp, M. N., Volkmann, R. A., Holland, G. F., Eggler, J. F., Clark, D. A., McPherson, R. K., Hall, K. L., Danbury, B. H., Gibbs, E. M., and Kreutter, D. K. (1990) *Diabetes* **39,** 1218–1227
10. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkinson, W. O., Wilson, T. M., and Kliewer, S. A. (1995) *J. Biol. Chem.* **270,** 12953–12956
11. Kletzien, R. F., Clarke, S. D., and Ulrich, R. G. (1992) *Mol. Pharmacol.* **41,** 393–398
12. Sandouk, T., Reda, D., and Hofmann, C. (1993) *Am. J. Physiol.* **264,** C1600–C1608
13. Tafuri, S. R. (1996) *Endocrinology* **137,** 4706–4712
14. Schoonjans, K., Peinadoonsurbe, J., Lefebvre, A. M., Heyman, R. A., Briggs, M., Deeb, S., Staels, B., and Auwerx, J. (1996) *EMBO J.* **15,** 5336–5348
15. Clancy, B. M., and Czech, M. P. (1990) *J. Biol. Chem.* **265,** 12434–12443
16. Lima, F. B., Bao, S., and Garvey, W. T. (1994) *Diabetes* **43,** 53–62
17. Kern, P. A., Marshall, S., and Eckel, R. H. (1985) *J. Clin. Invest.* **75,** 199–208
18. Nilsson-Ehle, P., and Schotz, M. C. (1976) *J. Lipid Res.* **17,** 536–541
19. Belfrage, P., and Vaughn, M. (1969) *J. Lipid Res.* **10,** 341–344
20. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159
21. Ong, J. M., Kirchgessner, T. G., Schotz, M. C., and Kern, P. A. (1988) *J. Biol. Chem.* **263,** 12933–12938
22. Szalkowski, D., White-Carrington, S., Berger, J., and Zhang, B. (1995) *Endocrinology* **136,** 1474–1481
23. Spiegelman, B. M., and Flier, J. S. (1996) *Cell* **87,** 377–389
24. Brun, R. P., Kim, J. B., Hu, E., Altiok, S., and Spiegelman, B. M. (1996) *Curr. Opin. Cell Biol.* **8,** 826–832
25. Enerbäck, S., and Gimble, J. M. (1993) *Biochim. Biophys. Acta* **1169,** 107–125
26. Doolittle, M. H., Ben-Zeev, O., Elovson, J., Martin, D., and Kirchgessner, T. G. (1990) *J. Biol. Chem.* **265,** 4570–4577
27. Ong, J. M., and Kern, P. A. (1989) *J. Clin. Invest.* **84,** 305–311
28. Yukht, A., Davis, R. C., Ong, J. M., Ranganathan, G., and Kern, P. A. (1995) *J. Clin. Invest.* **96,** 2438–2444
29. Semenkovich, C. F., Wims, M., Noe, L., Etienne, J., and Chan, L. (1989) *J. Biol. Chem.* **264,** 9030–9038
30. Ben-Zeev, O., Doolittle, M. H., Davis, R. C., Elovson, J., and Schotz, M. C. (1992) *J. Biol. Chem.* **267,** 6219–6227
31. Simsolo, R. B., Ong, J. M., and Kern, P. A. (1992) *J. Lipid Res.* **32,** 1777–1784
32. Olivecrona, T., Chernick, S. S., Bengtsson-Olivecrona, G., Garrison, M., and Scow, R. O. (1987) *J. Biol. Chem.* **262,** 10748–10759
33. Fulgencio, J.-P., Kohl, C., Girard, G., and Pegorier, J.-P. (1996) *Diabetes* **45,** 1556–1562
34. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366
35. Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvre, A., Saladin, R., Najib, J., Laville, M., Fruchart, J.-C., Deeb, S., Vidal-Puig, V., Flier, J., Briggs, M. R., Staels, B., Vidal, H., and Auwerx, J. (1997) *J. Biol. Chem.* **272,** 18779–18789
36. Cominacini, L., Garbin, U., Pastorino, A. M., Campagnola, M., Fratta Pasini, A., Davoli, A., Rigoni, A., and Lo Cascio, V. (1997) *Diabetologia* **40,** 165–172
37. Law, R. E., Meehan, W. P., Xi, X.-P., Graf, K., Wuthrich, D. A., Coats, W., and Faxon, D. (1996) *J. Clin. Invest.* **98,** 1897–1905
38. Lefebvre, A.-M., Peinado-Onsurbe, J., Leitersdorf, I., Briggs, M. R., Paterniti, J. R., Fruchart, J. C., Fievet, C., Auwerx, J., and Staels, B. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17,** 1756–1764
39. Pfeifer, M. A., Brunzell, J. D., Best, J. D., Judzewitsch, R. G., Halter, J. B., and Porte, D., Jr. (1983) *Diabetes* **32,** 525–531
40. Pykalisto, O., Smith, P. H., and Brunzell, J. D. (1975) *J. Clin. Invest.* **56,** 1108–1116
41. Bierman, E. L. (1992) *Arterioscler. Thromb.* **12,** 647–656
42. Simsolo, R. B., Ong, J. M., Saffari, B., and Kern, P. A. (1992) *J. Lipid Res.* **33,** 89–95
43. Burant, C. F., Sreenan, S., Hirano, K., Tai, T.-A. C., Ohmiller, J., Lukens, J., Davidson, N. O., Ross, S., and Graves, R. A. (1997) *J. Clin. Invest.* **100,** 2900–2908