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Regulation of adipose triglyceride lipase by rosiglitazone

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Abstract

Aim—To elucidate the mechanism by which rosiglitazone regulates adipose triglyceride lipase (ATGL).

Methods—Male C57Bl/6 mice were treated with rosiglitazone daily (10 mg/kg body weight), and adipose tissues were weighed and preserved for mRNA and protein analysis of ATGL. In parallel, preadipocyte (3T3-L1) cells were differentiated with insulin/dexamethasone/3-isobutyl-1-methylxanthine cocktail or rosiglitazone, and ATGL levels were measured with real-time PCR, western blotting and immunohistochemistry.

Results—Rosiglitazone concomitantly promoted differentiation of pre-adipocytes to functional adipocytes and induced mRNA levels of ATGL. The peroxisome proliferator-activated receptor- (PPAR) antagonist bisphenol A diglycidyl ether significantly abrogated the induction of mRNA, but not protein levels of ATGL by rosiglitazone in differentiated 3T3-L1 adipocytes. In the presence of epinephrine rosiglitazone stimulated free fatty acid release and increased diacylglycerol acyltransferase-1 (DGAT-1) mRNA suggest that ATGL and DGAT-1 may be cooperatively involved in rosiglitazone-stimulated triglyceride hydrolysis and fatty acid re-esterification in 3T3-L1 adipocytes. Treatment of 3T3-L1 adipocytes with rosiglitazone or insulin did not appear to alter localization of ATGL staining surrounding lipid droplets. Finally, we found that rosiglitazone increased ATGL mRNA levels in 3T3-L1 adipocytes in the presence of cycloheximide, an inhibitor of protein synthesis, suggesting that rosiglitazone regulation of ATGL occurs at the transcriptional level.

Conclusions—Rosiglitazone directly regulates transcription of ATGL, likely through a PPAR - mediated mechanism.

Keywords

adipocyte; ATGL; PPAR ; rosiglitazone

Introduction

Adipose tissue is an important source of energy and plays an important role in regulating energy homeostasis by governing the mobilization of fat from white adipose tissues. In adipose tissue, lipolysis is tightly controlled by hormones such as insulin and

catecholamines. During fasting or starvation, release of free fatty acids (FAAs) from hydrolysis of triacylglyceride (TAG) is increased in response to activated hormone-sensitive lipase (HSL) by catechol-amines mediated through the cAMP/PKA pathway [1]. After a meal, HSL is acutely suppressed by insulin thereby inhibiting lipolysis. However, a previous report demonstrated increased diacylglycerol (DAG) but not TAG accumulation in the adipose of HSL null mice [2], suggesting that HSL is not responsible for the hydrolysis of TAG to DAG, and there was likely another lipase involved in the hydrolysis of TAG in adipose tissue [2].

Adipose triglyceride lipase (ATGL/desnulin/calcium-independent phospholipase A2) is a recently discovered TAG lipase that hydrolyzes the first ester bond of TAG to generate DAG and FFA in mouse adipose tissue [3-5]. ATGL is critical for the hydrolysis of TG in adipose tissue in animals during basal lipolysis [6]. The importance of ATGL in adipocyte lipid metabolism is supported by the evidence that mice lacking ATGL exhibited an increase in adipose mass and deposition of TAG in adipose tissues [3-5,7]. Current literature indicates that ATGL plays a major role in TAG hydrolysis in murine adipocytes. The role of ATGL in human lipolysis of adipocytes seems less understood than in animals. The expression of ATGL in humans is associated with insulin resistance but not closely related to obesity [8,9]. The mechanism of regulation of ATGL remains unclear but is upregulated during adipocyte differentiation and fasting [5] and downregulated by tumor necrosis factor- α , iso-proterenol and insulin [10,11]. A recent report indicated that rosiglitazone, a peroxisome proliferator-activated receptor- γ (PPAR γ) agonist, induced lipolysis and increased mRNA levels of ATGL and fatty acid recycling in rat adipose tissues [12].

The mechanism for regulation of ATGL mRNA and protein synthesis by PPAR γ agonist rosiglitazone is not fully understood. At least part of the insulin-sensitizing action of rosiglitazone may occur through glyceroneogenesis pathway in adipose tissue [13,14]. Overexpression of diacylglycerol acyltransferase (DGAT-1), the terminal acyltransferase in the final step of TAG synthesis, resulted in enhanced re-esterification rates and increased adipose tissue mass [15]. Recent evidence shows that PPAR γ agonist increased expression of genes involved in TAG hydrolysis (ATGL) and fatty acid esterification (DGAT-1) [16]. Induction of ATGL by rosiglitazone induces a futile metabolic cycle in adipocytes that is associated with a simultaneous increase in TAG hydrolysis and synthesis. This study tests the hypotheses that (i) inhibition of PPAR γ decreases the induction of ATGL by rosiglitazone and (ii) ATGL is a direct target of rosiglitazone at transcriptional levels.

Research Design and Methods

Animal Study

Four-week-old, male C57Bl/6 mice were obtained from Harlan (Indianapolis, IN, USA) and housed four to five per cage at 22 ± 0.5 °C on a 12-h day/night cycle. Mice received standard mouse chow for 2 weeks while adjusting to their new environment and then were randomly assigned to two groups Vehicle control (CON) or Rosiglitazone (ROSI) (n = 10/group). After 3 weeks on a high-fat diet (45 kcal % as fat, 35% kcal as carbohydrate and 20% kcal as protein; Research Diet, Inc), mice received intraperitoneal injections of either 10 mg/kg body weight rosiglitazone (Cayman Chemical, Ann Arbor, MI, USA) (ROSI group) or a similar volume of the vehicle (phosphate-buffered saline, PBS) (CON group) daily for 6 weeks. Mice remained on the high-fat diet during the treatment period. Body weight, food intake and serum metabolites are shown in table 1. After 6 weeks of treatment, animals were anaesthetized by isoflurane, and blood was collected by cardiac heart puncture. Epididymal adipose tissues were isolated and immediately snap-frozen in liquid nitrogen. Serum samples were stored at -80 °C until analyses. All procedures were in

accordance with institution guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University.

Adipocyte Differentiation and Treatment

3T3-L1 cells (American Type Culture Collection) were grown for 2 days postconfluence in Dulbecco's modified Eagles medium (DMEM) containing glucose 4800 mg/l, 10% fetal bovine serum (FBS). Cells were induced to differentiate by changing medium to DMEM containing 10% FBS and either a cocktail (IDX) containing 10 µg/ml bovine insulin, 1 µM dexamethasone and 0.5 mM 3-iso-butyl-1-methylxanthine (IBMX; Sigma, St.Louis, MO) or rosiglitazone (1 µM) at day 0. Media containing differentiation cocktail was replaced with DMEM containing 10% FBS at day 2. Five days after induction of differentiation (day 5), approximately 80–90% of the cells were differentiated as determined by lipid accumulation and cell morphology by a phase-contrast microscope (data not shown). For studies on regulation of ATGL expression by PPAR antagonist/agonist during differentiation, PPAR antagonist Bisphenol A diglycidyl ether (BADGE; Caymen Chemicals) was added 24 h prior to induction of differentiation by rosiglitazone. Cells treated with rosiglitazone with or without pretreatment with BADGE were harvested at day 0, 2, 4 and 6. For studies on regulation of ATGL by PPAR antagonist/agonist, fully differentiated adipocytes were pretreated with BADGE (100 µM) 1 h prior to the addition of rosiglitazone (1 µM) at day 8. For studies designed to investigate the role of protein synthesis in induction of ATGL by rosiglitazone, cells were pretreated with the protein synthesis inhibitor cycloheximide (CHX) for 1 h then stimulated with or without rosiglitazone for 24 h.

Lipolysis and Protein Assays

For basal and stimulated lipolysis, differentiated 3T3-L1 adipocytes were serum starved for 3 h, washed twice with DMEM serum-free media and then incubated for 2 h in serum-free media containing 1% fatty acid-free bovine serum albumin in the presence or absence of epinephrine (100 nM). Glycerol and FFA content of conditioned medium was determined using GPO-Trinder Reagent (Sigma Chemicals, St Louis, MO, USA) and the NEFA-C kit (Wako Chemicals, Richmond, VA, USA) respectively. Final concentration of FFA and glycerol in the media was normalized by the protein content of cell lysates. The protein content of cell lysate was determined using the bicinchoninic acid protein assay kit (Pierce Chemicals, Rockford, IL, USA).

Real-time PCR Analysis

Total RNA from epididymal adipose tissues was isolated using an RNeasy Lipid Tissue kit (Qiagen, Valencia, CA, USA). Expression of RNA levels was analyzed by quantitative real-time PCR (Prism 7300 Sequence Detection System; Applied Biosystems, Foster City, CA, USA). Total RNA was reversed transcribed with random hexamers using MultiScribe reverse transcriptase (Applied Biosystems). After cDNA synthesis, real-time PCR analysis was performed using predesigned primes and probes supplied by Applied Biosystems (TaqMan Gene Expression Assays, Applied Biosystems). Target gene expression was expressed as 2^{-C_t} using comparative C_T method and normalized to the expression of 18S ribosomal RNA and the CON group.

Western Blot Analysis

Protein (30 µg) of epididymal adipose tissues or 3T3-L1 adipocyte lysates were separated on 10% polyacrylamide sodium dodecyl sulphate gels and then transferred onto nitrocellulose membranes. ATGL and α -actin levels were determined by blotting with anti-ATGL (Cell Signaling Technology, Danvers, MA, USA) and anti- α -actin primary antibodies (Cell

Signaling Technology). Quantification was performed using KODAK IMAGE software. Immunoblots were performed twice in duplicate with one representative set presented.

Confocal Laser Microscopy

3T3-L1 pre-adipocytes were grown on two-well chamber slides (Fisher Scientific, PA, USA) to confluence and were introduced to differentiate 2 days after confluence. On day 10 of differentiation, differentiated 3T3-L1 adipocytes were serum starved for 3 h, and then treated with rosiglitazone 1 μ M in the presence or absence of epinephrine 100 nM for 2 h. Cells were fixed with 3.7% paraformaldehyde in PBS for 1 h at room temperature and permeabilized with 0.1% Triton X-100 in PBS for 15 min and then blocked in PBS containing 10% goat serum (Invitrogen, Carlsbad, CA, USA) for 2 h. Fixed cells were incubated with anti-ATGL antibody (Cell Signaling Technology) and co-stained with guinea pig antiperilipin A/B antibodies (Research Diagnostics, Concord, MA, USA) at a dilution of 1:400 in 5% blocking solution overnight at 4 °C. The next day, following 1 h incubation with Alexa 555 (red) and 488 (green)-conjugated secondary antibodies at a dilution of 1:800 at room temperature, the stained cells were washed three to four times with PBS and then mounted with Prolong Antifade solution (Molecular Probes, Eugene, OR, USA) and observed using a Zeiss 510 confocal laser-scanning microscope (Carl Zeiss, Thornwood, NY, USA). Images were processed using LSM 5 IMAGE software.

Statistical Analysis

All data are presented as mean $\pm$ standard error (s.e.). Comparisons were analysed using two-sample Student's *t*-tests or one way ANOVA as appropriate (MINITAB). Differences were considered significant at $p < 0.05$.

Results

Rosiglitazone Increased ATGL mRNA and Protein Levels in Mouse Adipose Tissue

Administration of rosiglitazone (10 mg/kg) for 6 weeks in mice fed high-fat diet lowered fasting insulin levels, enhanced serum adiponectin levels (table 1) and induced expressions of genes involved in fatty acid utilization and lipogenesis in adipose tissue. Interestingly, we observed that rosiglitazone induced a twofold increase in ATGL mRNA (figure 1A) and a 0.7-fold increase in the protein levels of ATGL (figure 1B), which was paralleled by a significant twofold increase in DGAT-1 gene expression (figure 1C) in white adipose tissue.

Rosiglitazone Increased Free Fatty Acid Release *in vitro*

Because rosiglitazone affects lipolytic gene expression (ATGL and HSL) *in vivo* and *in vitro*, we next aimed to determine whether the changes in ATGL mRNA levels by rosiglitazone were associated with stimulation of lipolysis. We measured glycerol and FFA levels in the culture medium as a measure of lipolysis. Chronic administration of rosiglitazone for 6 weeks in mice fed a high-fat diet did not significantly alter circulating FFA levels (0.59 ± 0.1 vs. 0.50 ± 0.06 mmol/l CON vs. rosiglitazone). Short-term treatment with rosiglitazone (2 h) resulted in a 20% increase in epinephrine-stimulated FFA release in fully differentiated 3T3L-1 adipocytes compared with cells stimulated with epinephrine without rosiglitazone (figure 2A). Basal FFA levels were not altered by rosiglitazone treatment alone. Glycerol release was not altered by rosiglitazone either in the presence or in the absence of epinephrine (3.6 ± 0.22 vs. 3.70 ± 0.40 mg/dl vehicle vs. rosiglitazone) (figure 2B).

Induction of ATGL mRNA by Rosiglitazone was Prevented by the PPAR γ Antagonist BADGE in Fully Differentiated 3T3-L1 Adipocytes and During Adipogenesis

The effect of BADGE, a PPAR γ antagonist, on ATGL mRNA levels in rosiglitazone-treated adipocytes was investigated to determine whether rosiglitazone-induced ATGL mRNA in 3T3-L1 adipocytes is through a PPAR γ -dependent mechanism. Treatment with rosiglitazone for 24 h induced a 25-fold increase in mRNA levels of ATGL in fully differentiated cells. Pretreatment with BADGE resulted in a 50% decrease in ATGL mRNA in ROSI-treated cells (figure 2C).

Rosiglitazone-induced ATGL mRNA in 3T3-L1 Adipocytes

3T3-L1 adipocytes differentiated by rosiglitazone were accompanied by a 3.8-fold increase in mRNA levels of ATGL at day 2, reaching maximum levels by day 4 (~78-fold), which were maintained at day 6 (figure 3A). ATGL expression remained high (day 2–6) in adipocytes differentiated with rosiglitazone in the absence of IDX (figure 3B). The mRNA levels of ATGL were significantly induced (4.8-fold) at day 2, reaching maximum levels by day 4 and declining at day 6 in adipocytes differentiated with IDX compared with undifferentiated cells (figure 3B). At day 6, a 40% suppression of mRNA levels of ATGL in rosiglitazone-treated cells was observed in the presence of IDX (figure 3B). In addition, BADGE pretreatment blocked induction of PPAR γ mRNA expression by rosiglitazone (75%) (figure 3C). DGAT-1 mRNA increased by rosiglitazone showed a similar trend observed in rosiglitazone-induced ATGL mRNA expression (figure 3D).

Regulation of ATGL mRNA and Protein Levels by Rosiglitazone

A time-course study indicated that induction of ATGL mRNA by rosiglitazone occurred at 3 h and reached maximal at 24 h in 3T3-L1 adipocytes (figure 4A). A time-course study in fully differentiated 3T3-L1 adipocytes revealed that there were no significant changes in ATGL protein in either vehicle- or rosiglitazone-treated cells between 0 and 6 h. ROSI significantly increased levels of ATGL protein at 24 h (figure 4B). Because ATGL protein was increased by the PPAR γ agonist, rosiglitazone, *in vivo* and *in vitro*, we examined the effects of the PPAR γ antagonist, BADGE and insulin on rosiglitazone-induced protein levels of ATGL. Interestingly, BADGE had no effect on rosiglitazone-induced ATGL protein expression (figure 5A). Insulin was used here as a control because of its antilipolytic effect. The effects of rosiglitazone on ATGL protein expression were investigated in the absence and the presence of insulin in 3T3-L1 adipocyte. Here, we showed that insulin significantly suppressed protein levels of ATGL in either vehicle- or rosiglitazone-treated cells (figure 5B).

Rosiglitazone Enhanced Endogenous ATGL in Adipocytes

As previously reported, perilipin and ATGL are essential components of the lipolysis pathway, yet the molecular mechanism leading to ATGL activation remains to be elucidated. Perilipin A was located abundantly on the surface of lipid droplets and protects TAG storage from HSL-mediated lipolysis. Direct interaction of ATGL and perilipin on lipid droplets is unknown. Therefore, we examined the ATGL localization in rosiglitazone treated cells in the presence or the absence of epinephrine by an indirect immunofluorescence analysis. As shown in figure 6A, panel C treatment with rosiglitazone was associated with staining of endogenous ATGL surrounding the intracellular lipid droplets. Treatment with insulin showed a similar reduced staining of ATGL on lipid droplets figure 6A, panel B. To determine the co-localization of perilipin and ATGL in response to rosiglitazone treatment in the presence of lipolytic stimulation with epinephrine, we performed double-label immunofluorescence as shown in figure 6B; ATGL and perilipin co-localized on the lipid droplets in rosiglitazone-treated cells in the absence of epinephrine.

In the presence of epinephrine, staining ATGL and perilipin in rosiglitazone-treated cells appeared less associated on lipid droplets. These results are consistent with ATGL protein co-localizing with lipid droplets in the presence of rosiglitazone as shown in figure 6A, panel C.

As a PPAR ligand, rosiglitazone increases adipogenic genes during adipocyte differentiation. To understand the pleiotropic effects of rosiglitazone, we tested whether rosiglitazone directly regulates ATGL mRNA synthesis. If mRNA levels of ATGL are increased by rosiglitazone in the presence of CHX, a protein synthesis inhibitor, ATGL is likely to be a direct target of rosiglitazone. The mRNA levels of ATGL induced by rosiglitazone increased compared with the control regardless of the presence of CHX (figure 7A). As a control experiment, the effect of CHX (10 μ M) on protein synthesis of ATGL was detected by western blotting. CHX (10 μ M) alone or with rosiglitazone (1 μ M) completely blocked induction of ATGL protein in differentiated 3T3-L1 adipocytes (data not shown). A similar pattern of HSL mRNA levels in response to rosiglitazone with or without CHX was observed in this study (figure 7B).

Discussion

This study provides evidence that chronic rosiglitazone treatment (*in vivo*) increased mRNA and protein levels of ATGL in epididymal adipose tissues of C57BL/6 mice fed a high-fat diet. Furthermore, ATGL mRNA and protein levels were markedly induced in rosiglitazone-treated 3T3-L1 adipocytes. This effect was suppressed by the PPAR antagonist BADGE. In addition, we observed that rosiglitazone did not dramatically alter the localization of ATGL protein in lipid droplets. These data suggest that levels of ATGL are regulated by rosiglitazone at the transcriptional and/or post-transcriptional levels.

As an insulin sensitizer [17], rosiglitazone improves hyperglycaemia and insulin resistance. The antidiabetic action of rosiglitazone has been attributed to reduced circulating FAA levels in type 2 diabetes [18]. A defect in HSL expression likely plays a major role in the impaired lipolysis observed in obese subjects [19]. Until recently, the major enzyme responsible for lipolysis (mobilization of fat from adipose tissue) was thought to be HSL. Induction of HSL gene expression by rosiglitazone has been explained by the identification of a PPAR-responsive element in the mouse HSL promoter region [20,21]. Studies in HSL null mice highlighted the importance of other lipases in TAG hydrolysis in adipocytes. HSL-deficient mice exhibited decreased fatty acid esterification in WAT [22]. The role of PPAR in the regulation of lipolysis produced inconclusive results *in vivo* and *in vitro* [12,18,23]. However, we and others reported rosiglitazone increased mRNA levels of ATGL, HSL and DGAT-1 in rodent adipose tissue [12,24,25]. These observations led us to test the hypothesis that ATGL may be associated with rosiglitazone-mediated FAA release in adipose tissue.

Administration of rosiglitazone (15 mg/kg) for 7 days significantly increased weight gain and fat deposition with significant increases in both basal and stimulated lipolysis in rat adipose tissue [12]. In the current study, FFA levels were not altered by rosiglitazone *in vivo* (table 1). The discrepancy between the lipolysis-stimulated with rosiglitazone in the previous report [12] and the lack of effect of rosiglitazone on FFA release in our observations *in vivo* is likely because of (i) differences in doses of rosiglitazone used (15 vs. 10 mg/kg), (ii) mice fed high-fat vs. rats fed low-fat diets making turnover of FFAs different [12] or (iii) experimental protocol differences where our *in vivo* FFAs data may not represent lipolysis because lipolysis activity was not measured *per se* in our animal study. We did, however, observe acute FFA release stimulated by rosiglitazone in the presence of epinephrine in 3T3-L1 adipocytes. Rosiglitazone appears to influence ATGL expression, thereby increasing acute FFA release *in vitro*. Concomitant with increases in FFA release

and ATGL, rosiglitazone-induced mRNA levels of DGAT-1 suggest that the released fatty acids might be taken up by adipocytes for re-esterification. Our data, along with previous findings [12] suggest that the expression of ATGL in the adipose tissue may participate in a futile metabolic cycle promoted by rosiglitazone.

The transcriptional regulation of ATGL is likely to be mediated, in part, through a PPAR γ -dependent mechanism. Treatment of fully differentiated 3T3-L1 cells with BADGE suppressed rosiglitazone-induced ATGL mRNA by approximately 50%. Rosiglitazone induced a rapid induction of ATGL mRNA at 12 h in rat fat explants [12]. Our study revealed a rapid induction of ATGL mRNA expression *in vitro* by rosiglitazone after a relatively short time (3 h). In addition, the protein synthesis inhibitor cycloheximide had no effect on rosiglitazone-induced ATGL mRNA expression. Furthermore, our findings revealed a time-dependent effect of the PPAR γ agonist rosiglitazone on ATGL mRNA expression. This observation clearly indicated that ATGL is a target of rosiglitazone at the transcriptional and/or post-transcriptional level. However, future studies are needed to determine whether rosiglitazone increases ATGL enzyme activity and to confirm that PPAR γ mediates rosiglitazone-induced ATGL transcription using a chromatin immunoprecipitation assay.

BADGE, a synthetic antagonist of PPAR γ , inhibits PPAR γ transcriptional activity by approximately 70% [26]. BADGE also blocks rosiglitazone-induced transactivation of PPAR γ /RXR α and repress the expression of PPAR γ -responsive genes thus preventing differentiation and adipogenesis *in vitro* [26]. Interestingly, BADGE had no effect on rosiglitazone-induced ATGL protein levels at 24 h post-treatment. This suggests that the increased ATGL protein by rosiglitazone may be associated with the stabilization of ATGL protein, which might be regulated through a PPAR γ -independent mechanism. Immunostaining analyses of lipid droplets in 3T3-L1 adipocytes treated with rosiglitazone suggest that PPAR γ agonist impacts cellular localization ATGL protein. It is possible that localization of ATGL increased by rosiglitazone is associated with PPAR γ -mediated induction of ATGL transcription. However, whether PPAR γ regulates intracellular localization of ATGL during basal or hormone-stimulated lipolysis is not clear. Recent studies by Kershaw *et al.* [27], reported that induction of ATGL mRNA and protein by rosiglitazone is inhibited by a PPAR γ antagonist GW 9662 and is also reduced by siRNA knockdown of PPAR γ . These differing observations may be attributable to the duration of pretreatment of GW 9662 (12 h), and siRNA knockdown of PPAR γ (48 h) is different than we employed in this study.

On the protein level, both HSL and ATGL are phosphorylated, but ATGL phosphorylation has been shown to be independent of PKA, in contrast to HSL, which is a PKA substrate [3]. It is likely that the phosphorylation of ATGL protein affects its activity or stability. Therefore, it is possible that a post-translational modification of ATGL protein may be required for its activity and coordinately to regulate intracellular TAG hydrolysis.

While current knowledge shows that ATGL plays a key role in TAG hydrolysis by coordinating with HSL and perilipin in regulating adipocyte lipid metabolism [28], the requirement of increased protein synthesis or post-translational modifications of ATGL for regulation is unknown. CGI-58 (/ hydrolase domain containing protein-5) has been reported to regulate ATGL-mediated lipolysis [29]. A recent report indicated that rosiglitazone increases CGI-58 mRNA in white adipose tissue [25]. Therefore, rosiglitazone may be increasing ATGL activity through a concerted increase in both ATGL and CGI-58.

In summary, our findings suggest that ATGL is a direct target of rosiglitazone at transcriptional level. ATGL induction by PPAR γ ligand in adipocytes could be

physiologically significant. The induction of ATGL by rosiglitazone is likely associated with fat mobilization and, in turn, improves insulin sensitivity. To fully understand the mechanism by which PPAR regulates lipolysis in adipose tissues, future research is needed to investigate the interaction of ATGL with other lipid droplet associated proteins, for example HSL, CGI-58 and perilipin in the presence of PPAR agonist.

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Fig. 1.

Rosiglitazone upregulated expression of adipose triglyceride lipase (ATGL) mRNA and protein as well as diacylglycerol acyltransferase-1 (DGAT-1) mRNA in epididymal adipose tissue of mice. (A) ATGL mRNA. (B) ATGL protein. (C) DGAT-1 mRNA in epididymal adipose tissue of mice fed high-fat diet treated with rosiglitazone (10 mg/kg; rosiglitazone) or vehicle (control; CON) for 6 weeks. The protein levels of ATGL were measured by western blot analysis. Data were quantified by densitometry and normalized to β -actin. Each bar represents the mean $\pm$ s.e. ($n = 6-8$ mice per group). *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$).

Fig. 2.

Rosiglitazone increased free fatty acid (FFA) release and expression of adipose triglyceride lipase (ATGL) in adipocytes (A) FFA release and (B) glycerol in fully differentiated 3T3-L1 adipocytes stimulated with or without rosiglitazone (1 μ M) 2 h in the presence or absence of epinephrine (100 nM). FFA release from stimulated adipocytes was relatively compared with non-epinephrine stimulated cells. Data represent findings from three experiments. Values are averages of three replicates. (C) ATGL mRNA in fully differentiated 3T3-L1 adipocytes pretreated with or without bisphenol A diglycidyl ether (BADGE) (100 μ M) in the presence or absence of rosiglitazone (1 μ M) for 24 h. *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$) denoted by different superscripts.

Fig. 3.

Induction of adipose triglyceride lipase (ATGL) mRNA by rosiglitazone through peroxisome proliferator-activated receptor- (PPAR)-mediated mechanism. (A) ATGL mRNA in cells 3T3-L1 differentiated with rosiglitazone in the presence or absence of BADGE. (B) ATGL mRNA in cells differentiated with rosiglitazone in the presence or absence of IDX. (C) PPAR mRNA and (D) Diacylglycerol acyltransferase-1 (DGAT-1) mRNA in cell treated with rosiglitazone in the presence or absence of bisphenol A diglycidyl ether (BADGE). Data represent findings from three separate experiments. Values are averages of three replicates. *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$) within day denoted by different superscripts.

Fig. 4.

Time-course of effects of rosiglitazone on adipose triglyceride lipase (ATGL) mRNA and protein levels. (A) Time-course of rosiglitazone on ATGL mRNA levels. (B) Time-course of rosiglitazone on ATGL protein levels. The protein levels of ATGL were measured by western blot analysis. Data were quantified by densitometry and normalized to β -actin. Fully differentiated 3T3-L1 adipocytes (day 8) treated with or without rosiglitazone (1 μ M) as indicated time points. Data represent findings from three separate experiments. Values are averages of three replicates. *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$) denoted by different superscripts.

Fig. 5.

Lack of effect of peroxisome proliferator-activated receptor- antagonist on rosiglitazone-induced adipose triglyceride lipase (ATGL) protein. (A) ATGL protein in cells treated with or without rosiglitazone (1 μM) in the presence or absence of bisphenol A diglycidyl ether (BADGE) (100 μM). (B) ATGL protein in fully differentiated 3T3-L1 adipocytes treated with or without rosiglitazone (1 μM) for 24 h in the presence or absence of insulin (100 nM). The protein levels of ATGL were measured by western blot analysis. Data were quantified by densitometry and normalized to β -actin. Data are shown as relative ATGL protein compared with vehicle untreated cells. Data represent findings from three experiments. Values are averages of three replicates. *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$) denoted by different superscripts.

Fig. 6.

Adipose triglyceride lipase (ATGL) localizes with lipid droplet in the presence of rosiglitazone. (A) On day 10 of differentiation, 3T3-L1 cells were treated with (c) or without (a, b, d) rosiglitazone (1 μ M) for 6 h and cells were treated with (a) vehicle control (DMSO) or (b) insulin 100 nM for 6 h. Cells were fixed for 1 h in 3.7% paraformaldehyde and processed for immunofluorescence microscopy with anti-ATGL antibodies and Alexafluor 555-conjugated secondary antibodies. (B) Localization of perilipin and ATGL in rosiglitazone-treated cells. Differentiated 3T3-L1 cells treated with rosiglitazone 1 μ M in the presence or absence of epinephrine 100 nM for 2 h. Cell were fixed and incubated with anti-ATGL antibodies and co-stained with guinea pig antiperilipin antibodies overnight at 4°C.

Following 1-h incubation of Alexa 555- (red, ATGL) and 488- (green, perilipin) conjugated secondary antibodies, the stained cells were observed. Images were processed using LSM 5 IMAGE and IMAGE J software.

Fig. 7. Lack of effect of cycloheximide (CHX) on rosiglitazone-induced adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) mRNA. (A) ATGL and (B) HSL mRNA in 3T3-L1 adipocytes treated with or without rosiglitazone 1 μ M in the presence or absence of CHX (10 μ M) for 24 h. Data represent findings from three separate experiments. Values are averages of three replicates. *Values represent mean $\pm$ s.e. with significant differences ($p < 0.05$) denoted by different superscripts.

Table 1

Effects of rosiglitazone on food intake, body weight and physiological parameters

	CON	ROSI
Food intake (g/week)	29.0 ± 1.1	29.7 ± 2.9
Initial body weight (g) (week 0)	22.8 ± 0.6	22.4 ± 0.7
Final body weight (g) (week 6)	29.8 ± 0.6	30.8 ± 0.9
Liver (g)	1.05 ± 0.1	1.14 ± 0.1
Epididymal adipose (g)	1.24 ± 0.1	1.4 ± 0.2
Inguinal adipose (g)	0.59 ± 0.1	0.67 ± 0.1
Serum		
Triglyceride (mg/dl)	44.8 ± 4.2	30.3 ± 3.3
FFA (mmol/l)	0.59 ± 0.1	0.50 ± 0.06
Insulin (pg/ml)	851 ± 176	434 ± 45 [*]
Leptin (pg/ml)	7241 ± 1738	7347 ± 322
Adiponectin (μg/ml)	13.6 ± 2.7	24.2 ± 4.7 ^{**}

FFA, free fatty acid. Data are mean ± s.e. of 6 to 10 mice per treatment. Tissue weights and serum metabolites were determined after 6 weeks of rosiglitazone treatments. Initial body weight and final body weight were measured at week 0 and week 6. Serum metabolites were determined at fasted state.

^{*} $P < 0.05$ vs. CON,

^{**} $P < 0.01$ vs. CON.