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Peroxisome Proliferator-activated Receptor γ Is Not Associated with Adipogenesis in Female Mice

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The peroxisome proliferator-activated receptor γ (PPARγ) plays a central role in adipogenesis and lipid storage. The PPARγ ligands, thiazolidinediones (TZDs), enhance in vitro adipogenesis in several cell types, but the role of the TZDs on *in vivo* adipogenesis is still poorly understood. To investigate how PPARγ ligand troglitazone regulates adipogenesis in female mice, we examined the effects of the troglitazone on adipose tissue mass, morphological changes of adipocytes, and the expression of PPARγ target and adipocyte-specific genes in low fat diet-fed female C57BL/6 mice. Administration of troglitazone for 13 weeks did not change body and total white adipose tissue weights compared with control mice. Troglitazone treatment also did not cause a significant decrease in the average size of adipocytes in parametrial adipose tissue although it is reported to increase the number of small adipocytes in male animals. Troglitazone did not affect the mRNA expression of PPARγ and its target genes as well as adipocyte-specific genes in parametrial adipose tissue. These results suggest that PPARγ does not seem to be associated with adipogenesis in females with functioning ovaries and that its inability to induce adipogenesis may be due to sex-related factors.

Key Words: Troglitazone, PPARγ (peroxisome proliferator-activated receptor γ), Adipogenesis, Female, Sex

INTRODUCTION

Adipose tissue is now understood to play a central role in metabolic regulation, feeding behavior and secretion of a variety of metabolic hormones. Adipose tissue has considerable capacity to expand through a complex interplay between proliferation and differentiation of preadipocytes into functional adipocytes (adipogenesis), and an increase in individual adipocyte size (hypertrophy) (Bertrand et al., 1978).

Many aspects of adipogenesis could be described as a cascade of gene expression regulated by a transcription factors, including CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor γ (PPARγ) (Barak et al., 1999; Rosen et al., 2000).

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Activated PPARγ modulates target gene expression by binding to DNA response elements composed of a direct repeat of the hexameric core motif AGGTCA separated by a single base pair after heterodimerization with retinoid X receptor (RXR). The PPARγ/RXR heterodimer plays a crucial role in adipogenesis and insulin sensitization (Rosen and Spiegelman, 2000; Willson et al., 2001; Camp et al., 2002; Rangwala and Lazar, 2004). Previous studies have demonstrated that heterozygous PPARγ-deficient mice are protected from high-fat diet-induced adipocyte hypertrophy, obesity and insulin resistance (Kubota et al., 1999; Miles et al., 2000), suggesting that PPARγ may have a pivotal role in adipocyte hypertrophy and insulin resistance.

The thiazolidinediones (TZDs) are a class of antidiabetic drugs for the treatment of type II diabetes on the basis of their ability to lower glucose levels. TZDs mediate their therapeutic effects through direct interactions with PPARγ (Lehmann et al., 1995; Spiegelman, 1998; Hauner, 2002). Obese Zucker rats treated with troglitazone were improved in insulin sensitivity without adipose tissue mass gain (Okuno et al., 1998). Moreover, a key role for PPARγ on

adipogenesis is supported by evidences that synthetic PPARγ ligands regulate differentiation of a variable of cell types including 3T3-L1 preadipocytes (Lehmann et al., 1995; Oberfield et al., 1999; Wang et al., 2008). BRL49653 and rosiglitazone promoted differentiation of C3H10T1/2 cells to adipocytes, but GW0072 inhibited adipocyte differentiation. Troglitazone also enhanced lipid accumulation and expression of PPARγ-responsive genes during the differentiation of 3T3-L1 preadipocytes, suggesting that PPARγ activity is required for *in vitro* adipocyte differentiation. However, TZDs-activated PPARγ actions on *in vivo* adipogenesisis remains unclear although there were researches that TZDs-induced PPARγ activity modulates *in vitro* adipogenesis and improves *in vivo* insulin sensitivity.

Adipose tissue is also a target for sex steroids because gonadal sex steroid hormone receptors are expressed in rat and human adipose tissues (Pedersen et al., 1991; Mizutani et al., 1994). Particularly, estrogen plays an important role in white adipose tissue (WAT) regulation and adipogenesis. Ovariectomized (OVX) animals increased WAT whereas estrogen therapy decreased WAT levels compared with untreated OVX animals (Yoon et al., 2003; Jeong et al., 2004; Jeong and Yoon, 2007). Estrogen favored early osteogenic commitment and inhibited adipogensis of mouse ST2 cells overexpressing either estrogen receptor (ER) α or ERβ (Okazaki et al., 2002). Moreover, there are data indicating that PPARγ is capable of interacting with ER signal transduction pathway (Wang and Kilgore, 2002).

Accordingly, we decided to study the effects *in vivo* of troglitazone on adipogenesis in female mice. Here we report that troglitazone-activated PPARγ do not affect adipogenesis in females maybe due to its interaction with ovarian factors.

MATERIALS AND METHODS

1. Animal treatments

For all experiments, eight-week-old mice (C57BL/6) were housed and bred at the Korea Research Institute of Bioscience and Biotechnology under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Female mice were each randomly divided into two groups (n=5/group) and received either a low fat diet (4.5% fat, w/w, CJ, Korea) or the same low fat diet supplemented with troglitazone (0.2% w/w, Sankyo Co., Ltd., Tokyo, Japan) for 13 weeks. In all experiments, animals were sacrificed by cervical dislocation, tissues were harvested, weighed, snap frozen in liquid nitrogen and stored at -80℃ until use.

2. Histological analysis

For hematoxylin and eosin (HE) staining, female parameterial adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner for paraffin sections. Sections of thickness 5 μm were cut and stained with HE for light microscopic examination. To quantitate the number and size of adipocytes, the sectional areas of adipose tissues in the HE-stained

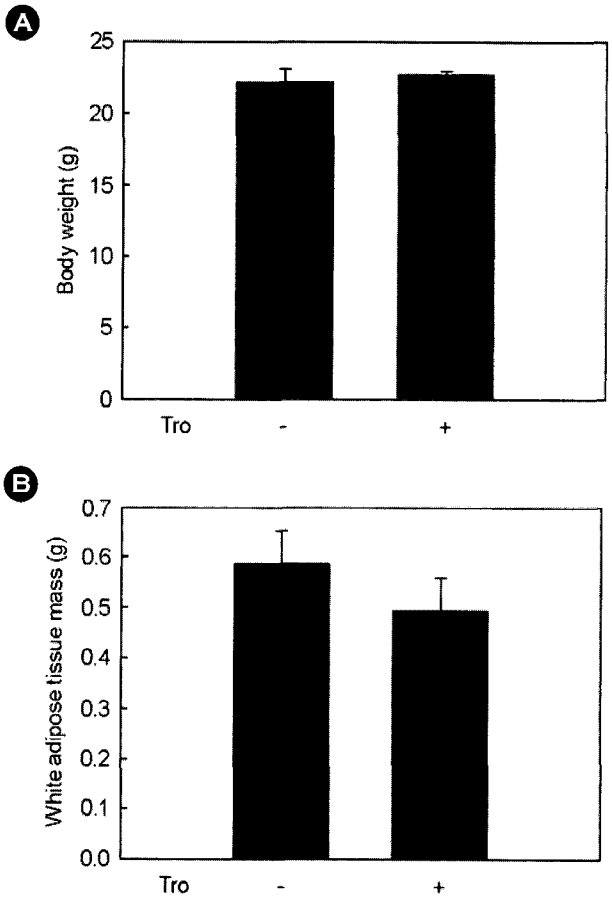

Fig. 1. Effects of troglitazone on body weight and white adipose tissue mass in female 57BL/6 mice. Adult female mice (n=5/group) received a low fat diet or the same low fat diet with troglitazone (Tro; 0.2% w/w) for 13 weeks.

preparations were analyzed with an image analysis system (Image Pro-Plus, Silver Spring, MD, USA).

3. Analysis of target gene expression

Total RNA was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY, USA) and analyzed by electrophoresis on 0.22 M formaldehyde-containing 1.2% agarose gels. The separated RNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Dassel, Germany) by downward capillary transfer in the presence of 20×SSC buffer (3 M NaCl, 0.3 M sodium citrate, pH 7.0), UV-crosslinked, and baked for 2 h at 80℃. Probe hybridization and washing were performed using standard techniques. Blots were exposed to phosphorimager screen cassettes and were visualized using a Molecular Dynamics Storm 860 PhosphorImager system (Sunnyvale, CA, USA). The probes used in this study were ^{32}P-labeled by the random-primer method using a Ready-to-Go DNA Labeling kit (Amersham-Pharmacia Biotech, Piscataway, NJ, USA), as previously described (Sinal et al., 2001). Densitometric analysis of the mRNA signals was performed using ImageQuant image analysis software (Molecular Dynamics, Sunnyvale, CA, USA).

4. Statistics

Unless otherwise noted, all values are expressed as mean ± standard deviation (SD). All data were analyzed by unpaired t-test for statistically significant differences between each group.

RESULTS

To determine whether troglitazone treatment regulates *in*

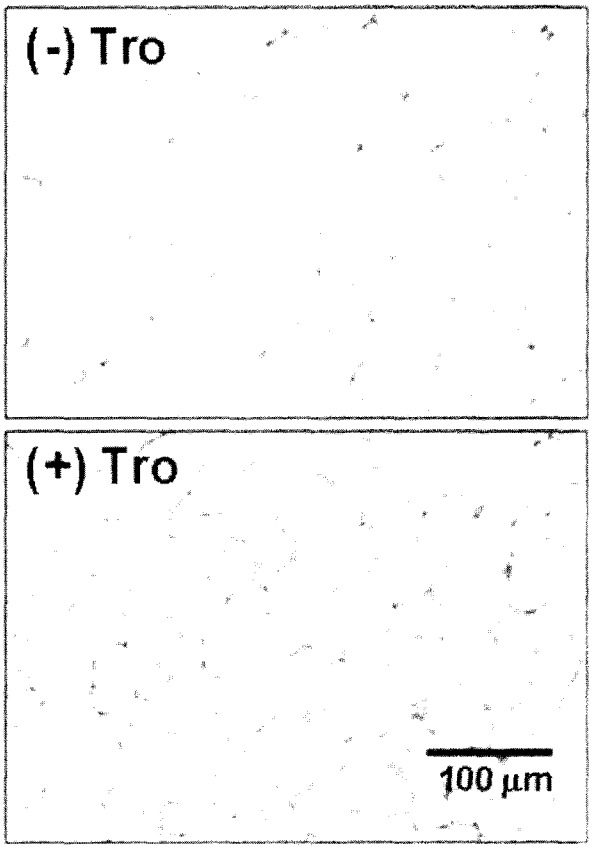

Fig. 2. Histology of parameterial adipose tissues stained with hematoxylin and eosin in female C57BL/6 mice (original magnification × 100). Adult female mice (n=5/group) received a low fat diet or the same low fat diet with troglitazone (Tro; 0.2% w/w) for 13 weeks. Shown are representative hematoxylin and eosin-stained sections (5 μm thick) of female parameterial adipose tissue.

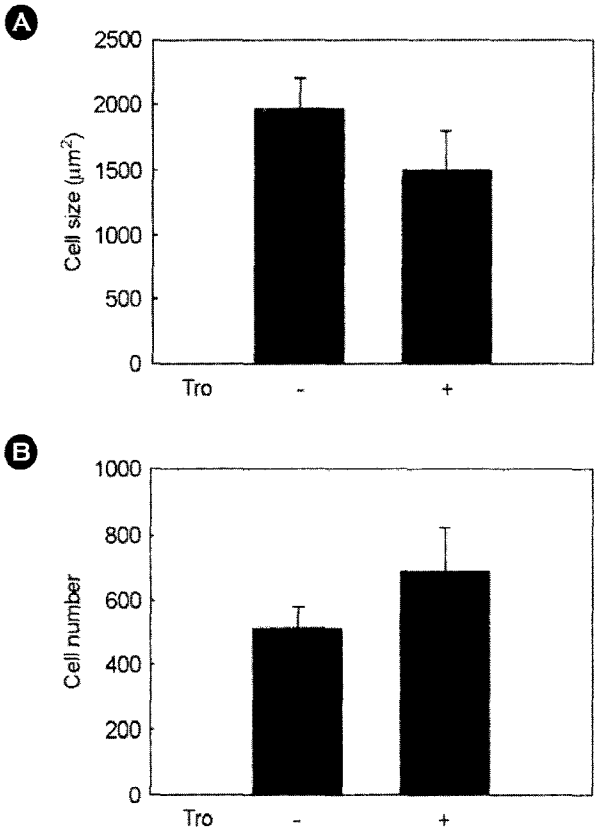

Fig. 3. Size and number of parameterial adipocytes in female C57BL/6 mice. Size of adipocytes and their numbers in a fixed area (1,000,000 μm^2) were quantified by an image analysis system. Female mice (n=5/group) received a low fat or the same low fat diet supplemented with troglitazone (Tro; 0.2% w/w) for 13 weeks. Size **(A)** and number **(B)** of adipocytes were measured and all values are expressed as the mean ± SD.

vivo adipogenesis in female mice, we have examined the changes in body weight and total WAT mass (Fig. 1). Administration of troglitazone for 13 weeks did not affect body weight in these animals. Similarly, total WAT mass was not significantly different between troglitazone-treated mice and control mice.

Adipogenesis includes morphological changes, cessation of cell growth, expression of many lipogenic enzymes and extensive lipid accumulation (Rosen and Spiegelman, 2000). To determine whether troglitazone regulates morphological changes of adipocytes in female mice, the size and number of parametrial adipocytes were measured (Figs. 2 and 3). In comparison with control mice, treatment of female mice with troglitazone did not cause a significant decrease in the average size of adipocytes in parametrial adipose tissue of female mice although a 23.9% decrease in adipoctye size was observed. Troglitazone showed a trend toward increasing the number of adipocytes in a fixed area, but did not exhibit a significant elevation.

We next studied the expression of PPARγ and adipocyte-specific PPARγ target genes involved in adipocyte differentiation, such as adipocyte protein 2 (aP2) and lipoprotein

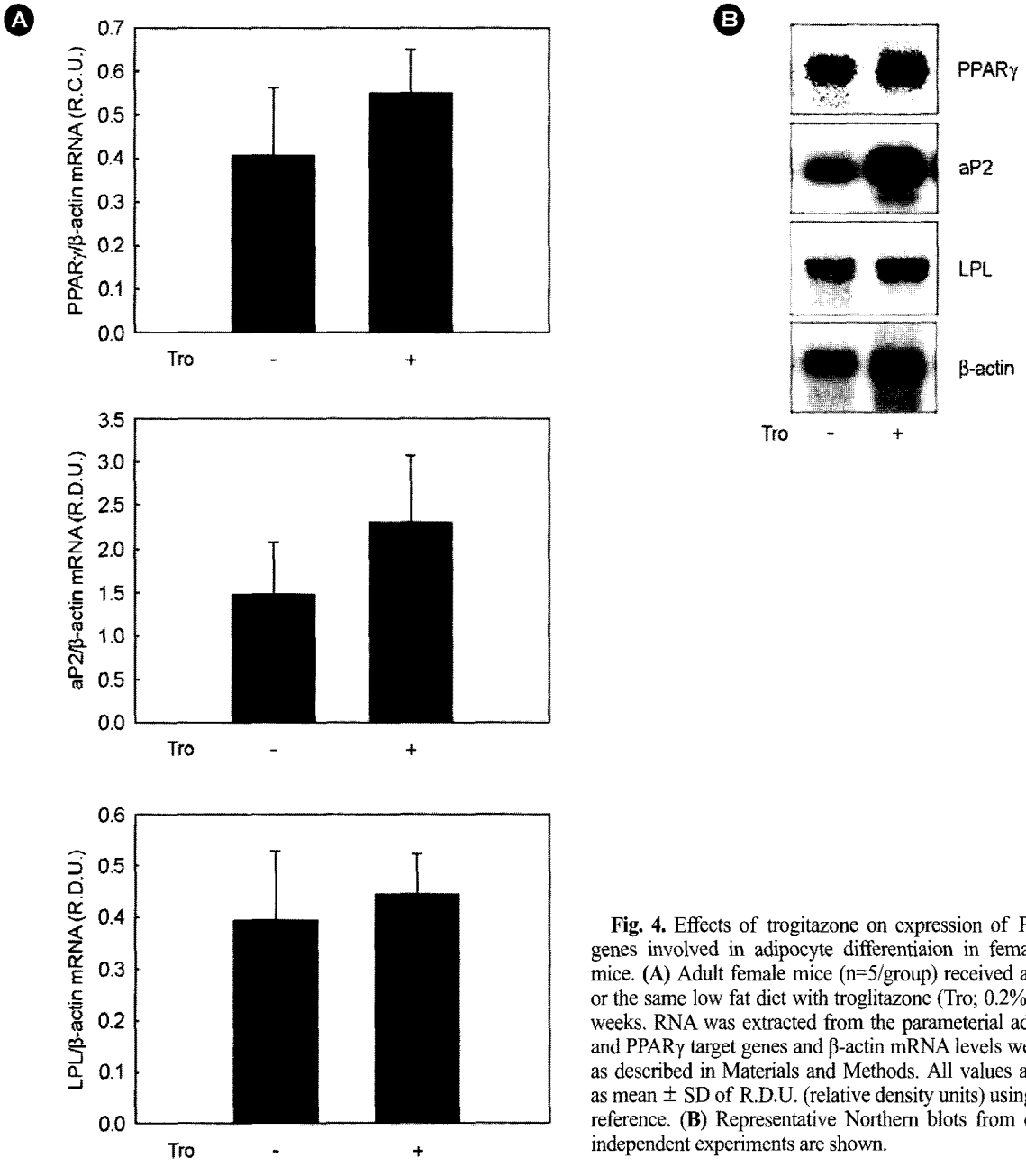

Fig. 4. Effects of trogitazone on expression of PPARγ target genes involved in adipocyte differentiaion in female C57BL/6 mice. **(A)** Adult female mice (n=5/group) received a low fat diet or the same low fat diet with troglitazone (Tro; 0.2% w/w) for 13 weeks. RNA was extracted from the parameterial adipose tissue, and PPARγ target genes and β-actin mRNA levels were measured as described in Materials and Methods. All values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin as a reference. **(B)** Representative Northern blots from one of three independent experiments are shown.

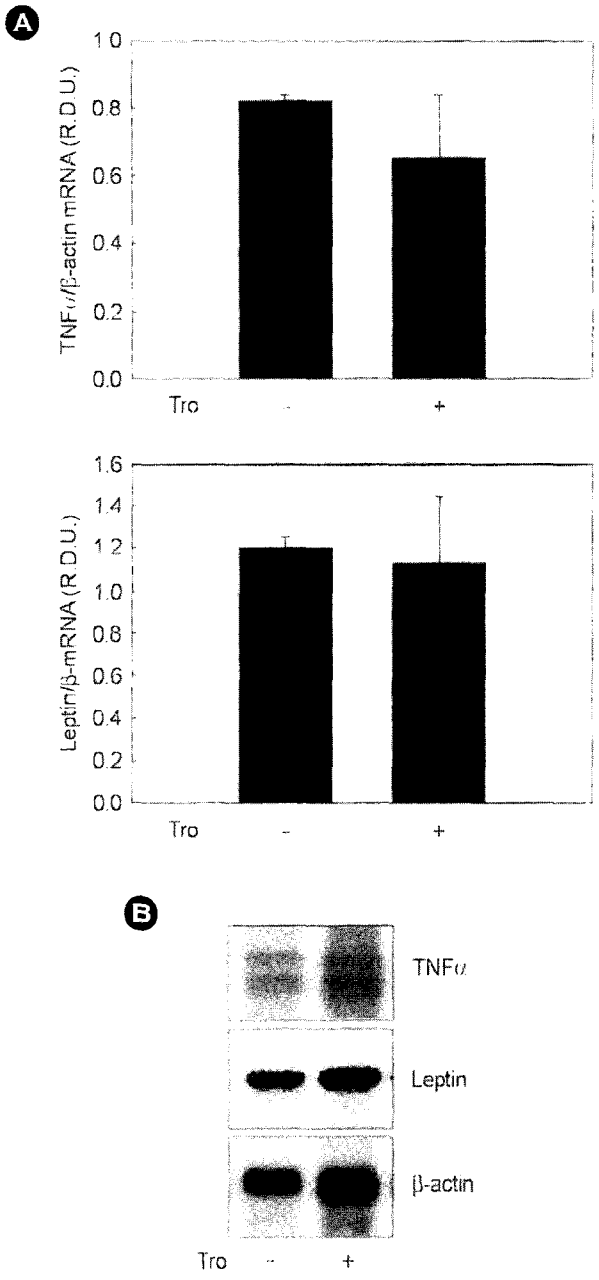

Fig. 5. Effects of trogitazone on expression of adipose marker genes in female C57BL/6 mice. **(A)** Adult female mice (n=5/group) received a low fat diet or the same low fat diet with troglitazone (Tro; 0.2% w/w) for 13 weeks. RNA was extracted from the parameterial adipose tissue, and adipocyte marker genes and β-actin mRNA levels were measured as described in Materials and Methods. All values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin as a reference. **(B)** Representative Northern blots from one of three independent experiments are shown.

lipase (LPL) in the parametrial adipose tissue (Fig. 4). Compared with control mice, troglitazone-treated female mice did not significantly increase the expression levels of PPARγ, aP2 and LPL by 35.6%, 55.8% and 12.7%, respectively, suggesting that troglitazone may not stimulate PPARγ actions in female mice. Consistent with the effects of troglitazone on adipocyte size, adipocyte marker genes such as tumor necrosis factor α (TNFα) and leptin mRNA levels were not significantly changed by troglitazone compared with control mice (Fig. 5).

DISCUSSION

Adipose tissue is also known to be a major target tissue of both PPARγ ligands TZDs and sex steroids that regulate WAT metabolism. Thus, this study was undertaken to investigate whether troglitazone regulates WAT metabolism in female mice with functioning ovaries.

Our results demonstrated that treatment of mice with troglitazone for 13 weeks did not exhibit a significant change in WAT mass as well as body weight. In contrast to data presented in this study, De Vos et al. (1996) reported the PPARγ ligand BRL49653 increased adipose tissue weight in adult male rats. Moreover, several lines of evidence using male animals showed that TZDs increased body weight gain and adipose tissue weight in an animal model of obesity and diabetes such as fatty Zucker rats and db/db mice (Wang et al., 1997; Chaput et al., 2000; de Souza et al., 2001). These results indicate that troglitazone may regulate body and adipose tissue weights with sexual dimorphism.

It was reported that the increase in adipose tissue mass was due to the enlargement of the preexisting adipocytes with increased lipid accumulation (Ogawa et al., 2004; Villena et al., 2004; Yagi et al., 2004). In parallel with the effect of WAT mass by troglitazone, morphological analysis showed that troglitazone treatment also did not cause significant changes in the average size of adipocytes as well as the number of adipocytes in parametrial WAT compared with control mice although troglitazone is known to decrease the adipocyte size and concomitantly increase the adipocyte number in a fixed area. According to Okuno et al. (1998), troglitazone increased the number of small adipocytes without the change of WAT mass through PPARγ actions in obese male Zucker rats. It is thus thought that troglitazone may differentially modulate adipocyte morphology between males and females.

Adipogenesis is initiated by the production of key tran-

scription factor PPARγ, which is responsible for inducing the expression of adipocyte-specific genes. We found that troglitazone did not have influences on PPARγ target gene expression, such as aP2 and LPL in WAT of female mice. In particular, 13 weeks of treatment with troglitazone did not increase the mRNA levels of aP2 and LPL responsible for fatty acid transport and lipoprotein catabolism, respectively, in WAT of females compared with controls. Moreover, leptin and TNFα are known to be markers for large adipocytes, as hypertrophic adipocytes in obesity increase generation or production of leptin and TNFα (Okuno et al., 1998; Zhang et al., 1994). However, troglitazone did not change mRNA expression of leptin and TNFα in our present study. Accordingly, our results indicate that troglitazone treatment do not induce PPARγ and adipocyte-specific gene expression in female mice.

Our present results suggest a possibility that the effects of troglitazone on adipogenesis may be interfered with sex-related factors in female mice. It was recently reported that estrogen decreased mRNA levels of aP2 and LPL in KS483 cells, indiciating that estrogen inhibits adipogenesis (Dang et al., 2002). It is more interesting that estrogen can modulate the adipogenic commitment of the mesenchymal mouse cell line KS483 via transcriptional repression of PPARγ. The phytoestrogen genistein also decreased the expression of PPARγ in 3T3-L1 cells (Harmon et al., 2002). Moreover, there are data indicating that signal cross talk exists bidirectionally between PPAR and estrogen receptor (ER) through competitive binding to DNA, competition for coactivators, and some other mechanism (Keller et al., 1995; Tcherepanova et al., 2000; Zhu et al., 2000; Wang and Kilgore, 2002; Jeong and Yoon, 2007). Thus, our results suggest that troglitazone may not affect the adipocyte size and adipose PPARγ actions in female mice maybe due to interaction between PPARγ and ER.

In conclusion, these data provide evidence that troglitazone treatment *in vivo* do not change adipose tissue mass and adipocyte size in WAT of female mice by inability to activate PPARγ. In addition, regulation of adipogenesis by troglitazone is likely to be influenced by sex-related factors.

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