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Troglitazone Lowers Serum Triglycerides with Sexual Dimorphism in C57BL/6J Mice

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Thiazolidinediones (TZDs) are widely used antidiabetic drugs that activate the nuclear peroxisome proliferator-activated receptor γ (PPARγ), and thereby improve the metabolic abnormalities linking hypertriglyceridemia to diabetes, hyperglycemia, insulin resistance, and cardiovascular disease. To determine whether the PPARγ ligand troglitazone regulates lipid metabolism with sexual dimorphism, we examined the effects of troglitazone on circulating lipids, body weight and the expression of hepatic genes responsible for lipid metabolism in both sexes of C57BL/6J mice. Compared to mice fed a low fat control diet, both sexes of mice fed a troglitazone-treated low fat diet for 14 weeks did not exhibit changes in body weight gain, serum total cholesterol, HDL-cholesterol and LDL-cholesterol levels. However, serum triglycerides were significantly reduced in both sexes of mice, although these effects were more pronounced among males. Furthermore, troglitazone regulated the expression of hepatic genes critical for lipid and lipoprotein metabolism, the magnitudes of which were much higher in males compared to females, as evidenced by results for increased acyl-CoA oxidase and decreased apolipoprotein C-III mRNA levels. These results suggest that PPARγ activator troglitazone may exert sexually dimorphic control of serum triglycerides in part through the differential activation of PPARγ in liver between male and female mice.

Key Words: PPARγ, Troglitazone, Triglycerides, Sex

INTRODUCTION

The peroxisome proliferator-activated receptors (PPARs) are members of a large family of ligand-activated transcription factors with essential roles in glucose and lipid metabolism (Rosen and Spiegelman, 2000; Debril et al., 2001; Wilson et al., 2001). Three genetically and functionally distinct PPAR isoforms occur in mammals, PPARα, PPARβ/δ, and PPARγ (Corton et al., 2000; Kersten et al., 2000; Kliewer et al., 2001). Ligand-bound PPARs modulate 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). PPAR · RXR heterodimers interact with PPAR response elements (PPREs) in the promoter region of target genes implicated in a number of physiological processes. PPARγ, in particular, exerts regulatory control over the expression of numerous genes encoding proteins involved in adipogenesis, insulin sensitization, and lipid metabolism (Spiegelman, 1998; Rosen et al., 2000; Way et al., 2001; Hauner, 2002).

Peroxisome proliferator-activated receptor γ (PPARγ) is the molecular target for the thiazolidinedione (TZD) family such as troglitazone, rosiglitazone, and pioglitazone (Lehmann et al., 1995; Furnsinn and Waldhausl, 2002). The TZDs were originally developed for the treatment of type II diabetes on the basis of its ability to lower glucose levels in rodent models of insulin resistance. These drugs also have beneficial effects on plasma levels of triglycerides that cause hypertriglyceridemia (Chaput et al., 2000). Hypertriglyceridemia is a risk factor for cardiovascular disease (Brewer, 1999). Activators of PPARγ are thus effective drugs to improve the metabolic abnormalities linking hypertriglyceridemia to diabetes, hyperglycemia, insulin resistance,

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and atherosclerosis. However, the mechanism of how TZDs work remains elusive.

Energy balance including lipid metabolism seems to differ, depending on the gonadal sex steroids (Mystkowski and Schwartz, 2000). Our previous studies have discussed that PPARα ligand fenofibrate reduced body weight gain and adiposity in male and ovariectomized female mice, but not in sham-operated female mice, indicating that the action of PPARα may be influenced by ovarian factors in females (Yoon et al., 2002, 2003; Jeong et al., 2004). The mechanism of actions of PPARγ is also similar to that of PPARα because both of them exert a number of physiological functions after binding to PPRE. PPARs and estrogen receptor (ER) bind to short DNA sequences termed hormone response elements, estrogen respone element (ERE) for ER and PPRE for PPARs (Klein-Hitpass et al., 1986; Tugwood et al., 1992). An ERE is an inverted repeat containing three intervening bases (AGGTCA N_3 TGACCT), whereas a PPRE is a direct repeat with a single intervening sequence (AGGTCA N AGGTCA). Nonetheless, these sequences contain an AGGTCA half site, which could be recognized by either ER or PPARs, suggesting that lipid metabolism by PPARγ can be regulated with sexual dimorphism.

Based on these previous reports, we suggest a possibility that the actions of the PPARγ ligand troglitazone in hepatic lipid metabolism may be regulated with sexual dimorphism. Here we report that troglitazone regulates circulating triglyceride metabolism in a sex-dependent manner, without the changes in body weight gain and serum cholesterol levels.

MATERIALS AND METHODS

1. Animal treatments

For all experiments, eight-week-old mice (C57BL/6J) 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*. Male and female mice were each randomly divided into two groups and received either a regular chow rodent diet (CJ Co., Korea) or a chow diet supplemented with troglitazone (0.2% w/w, Sankyo Co., Ltd., Tokyo, Japan) for 14 weeks. In all experiments, body weights were monitored throughout the treatment period. At the end of the study, blood samples were collected, from which serum was isolated and stored at -20℃ until further analysis. Animals were sacrificed by cervical dislocation, tissues were harvested, weighed, snap frozen in liquid nitrogen and stored at -80℃ until use.

2. Serum assays

Serum concentrations of total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol), and triglycerides were measured using an automatic blood chemical analyzer (CIBA corning, OH, USA).

3. Analysis of target gene expression

Total RNA was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY) and analyzed by electrophoresis on 0.22 M formaldehyde-containing 1.2% agarose gels. The separated RNA was transferred to Nytran membranes (Schneicher & 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). 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), as previously described (Sinal et al., 2001). Densitometric analysis of the mRNA signals was performed using ImageQuant image analysis software (Molecular Dynamics, Sunnyvale, CA).

4. Statistics

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

RESULTS

1. Effects of troglitazone on lipid levels

Since synthetic agonists of PPARγ are used in the treatment of metabolic diseases, such as dyslipidemia and type II diabetes, the effects of PPARγ ligand troglitazone on serum lipid levels were examined in both sexes of C57BL/

6J mice. Compared with respective control mice, serum triglycerides were significantly decreased in troglitazone-treated mice, by 52% in males ($P<0.01$) and by 21% in females ($P<0.01$) (Fig. 1). The extent of reductions in serum triglycerides was much higher in males than in females, showing a marked sexual dimorphism in PPARγ actions on circulating triglyceride metabolism. However, troglitazone treatment did not affect serum levels of total cholesterol, LDL-cholesterol, and HDL-cholesterol in both sexes of mice.

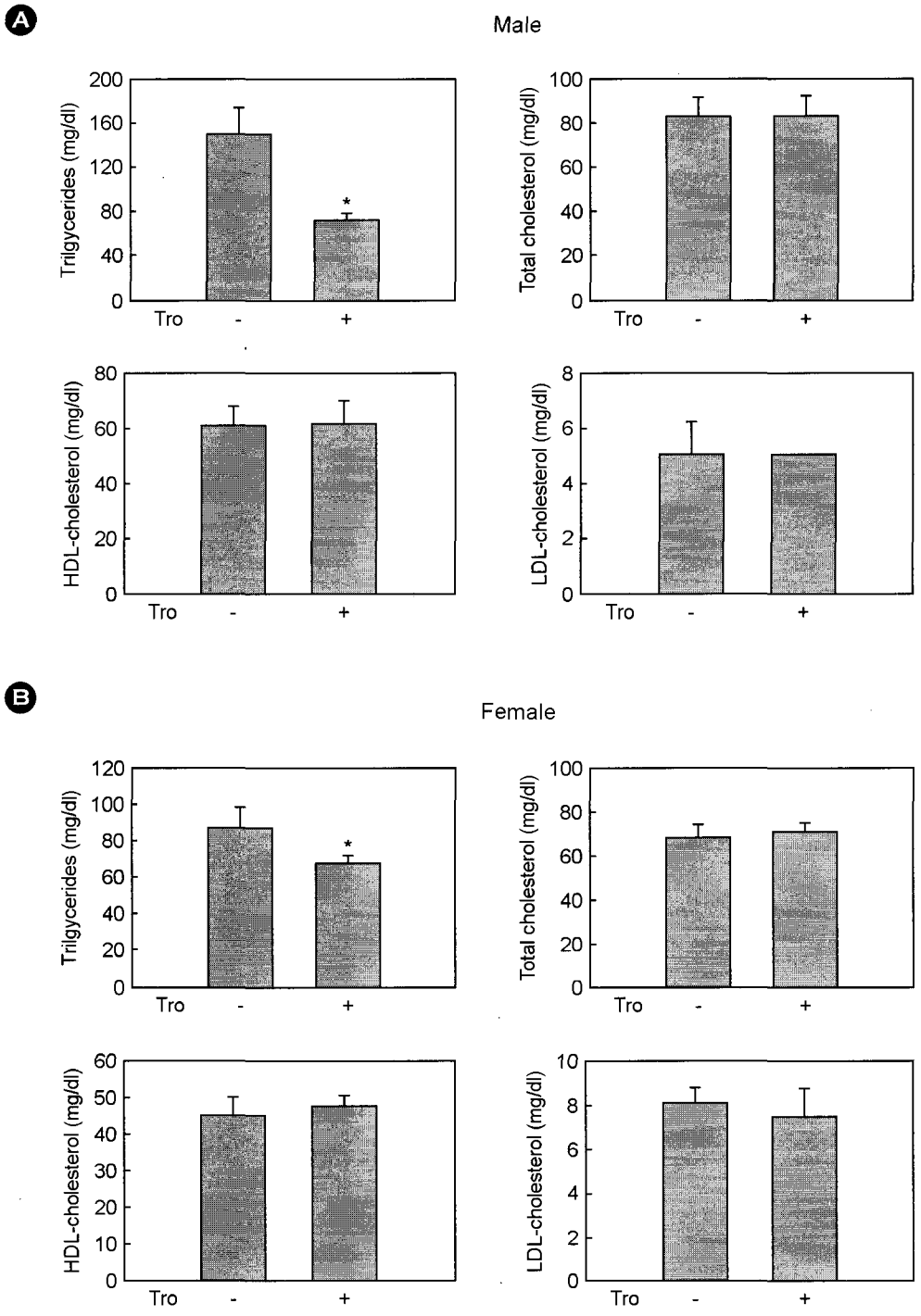

Fig. 1. The effects of troglitazone on circulating lipid levels in both sexes of C57BL/6J mice. **(A)** Male and **(B)** female C57BL/6J mice received a chow or the same chow diet supplemented with troglitazone (Tro; 0.2% w/w) for 14 weeks. Serum lipid levels were measured and all values are expressed as the mean ± SD. *, Significantly different versus low fat diet-only group, $P<0.01$.

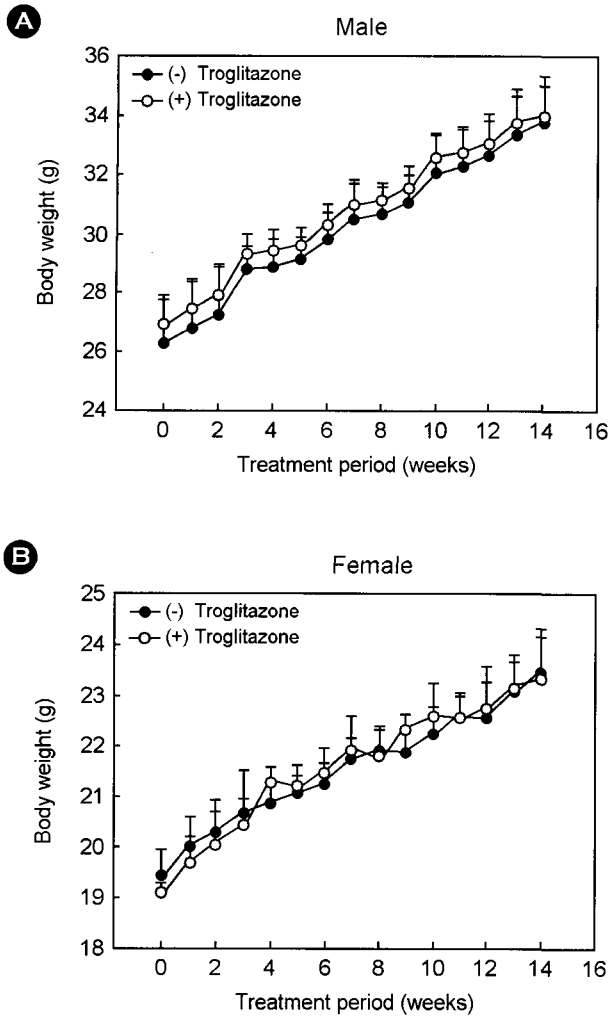

Fig. 2. The effects of troglitazone on body weight gain in both sexes of C57BL/6J mice. **(A)** Male and **(B)** female C57BL/6J mice received a chow or the same chow diet supplemented with troglitazone (0.2% w/w) for 14 weeks. All values are expressed as the mean ± SD.

2. Effects of troglitazone on body weight

Our previous data showed strong correlations among serum triglycerides, body weight and white adipose tissue (WAT) mass, which is supported by findings that WAT lipids are largely derived from serum triglycerides (Lupien et al., 1991; Yano et al., 1997; Fruchart et al., 1998). Thus, we examined whether decreased serum triglycerides are correlated with the changes in body weights in both sexes of mice. Compared to a respective chow diet, a troglitazone-treated chow diet did not decrease body weight gains in both sexes of C57BL/6J mice, although serum triglycerides were significantly decreased by troglitazone (Fig. 2).

3. Effects of troglitazone on hepatic ACOX and apo C-III mRNA levels

To evaluate whether the dimorphic effects of troglitazone on lipid profiles between male and female mice were caused by differential PPARγ actions in liver, we determined the expression of genes involved in the lipid metabolism, such as acyl-CoA oxidase (ACOX) and apolipoprotein C-III (apo C-III); these have crucial roles in the control of peroxisomal fatty acid β-oxidation system and in triglyceride metabolism, respectively. The troglitazone-containing chow diet-fed mice had elevated ACOX mRNA levels, by 321% in male ($P<0.001$) and by 78% in female mice ($P<0.05$) (Fig. 3). In response to troglitazone administration, apo C-III mRNA levels were significantly decreased in male mice, but not in female mice, compared with a chow diet alone ($P<0.05$). The apo C-III mRNA levels were 39% lower in the troglitazone-treated male mice than in their low fat diet-fed counterparts (Fig. 4). These results suggest that differences in troglitazone-mediated hepatic fatty acid β-oxidation and triglyceride metabolism may contribute to the differences in serum triglycerides between male and female animals.

DISCUSSION

This study was undertaken to verify whether PPARγ regulates lipid metabolism with sexual dimorphism and to get the information about the cellular mechanism involved.

Our results show that troglitazone substantially decreased circulating triglycerides in both sexes of mice through PPARγ actions in liver, without affecting the body weight gain, and that differences in triglyceride metabolism between male and females may be due to differential activation of PPARγ. We examined the effects of troglitazone on serum lipid levels in both sexes of C57BL/6J mice. Troglitazone treatment for 14 weeks caused decreases in serum triglycerides in both sexes of mice, while total cholesterol, HDL-cholesterol and LDL-cholesterol levels were not changed by troglitazone. These data are supported by other findings that TZDs decrease the circulating levels of triglycerides by inducing lipolysis and clearance of triglyceirde-rich lipoproteins in obese animal models (Stevenson et al., 1990; Kemnitz et al., 1994; Way et al., 2001). Interestingly, the magnitude of reductions in serum triglycerides was much

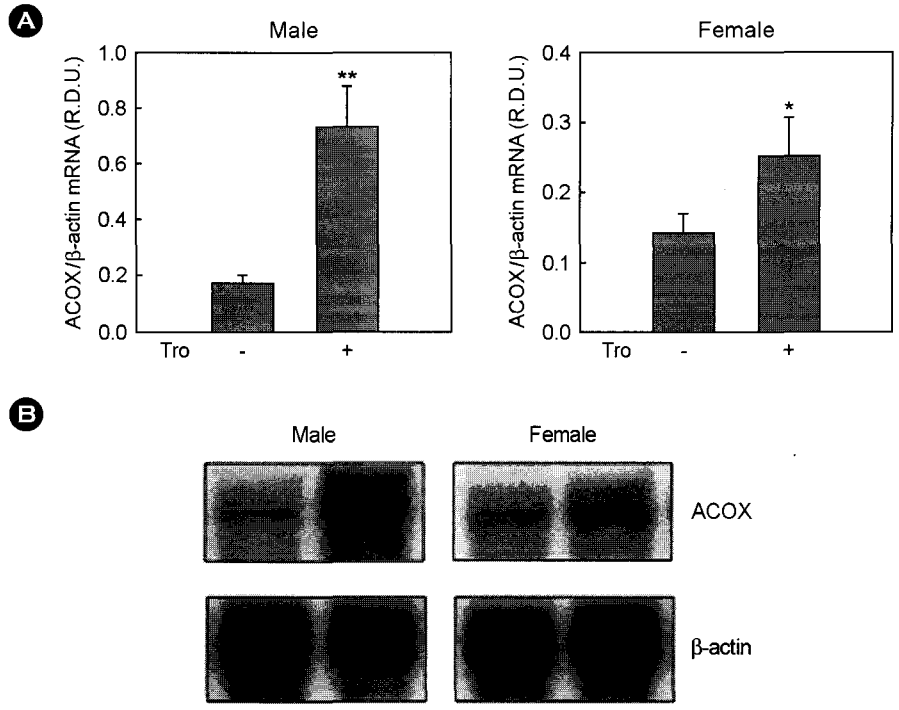

Fig. 3. The effects of troglitazone on ACOX mRNA levels in liver from both sexes of C57BL/6J mice. **(A)** Male and female C57BL/6J mice received a chow or the same chow diet supplemented with troglitazone (Tro; 0.2% w/w) for 14 weeks. RNA was extracted from liver and ACOX and β-actin mRNA levels were measured as described under "Methods". The mean ± SD for 3 animals is shown and all values are expressed in R.D.U. (relative density units) using β-actin as a reference. *, Significantly different versus chow diet-only group, $P<0.05$. **, Significantly different versus chow diet-only group, $P<0.001$. **(B)** Representative Northern blots, from an independent experiment.

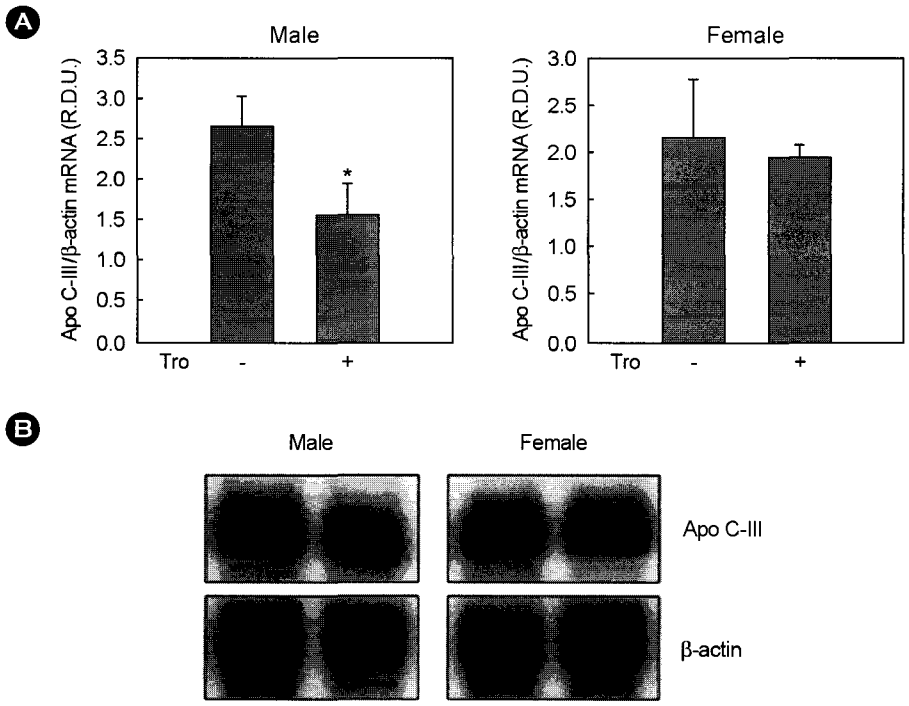

Fig. 4. The effects of troglitazone on apo C-III mRNA levels in liver from both sexes of C57BL/6J mice. **(A)** Male and female C57BL/6J mice received a chow or the same chow diet supplemented with troglitazone (Tro; 0.2% w/w) for 14 weeks. RNA was extracted from liver and apo C-III and β-actin mRNA levels were measured as described under "Methods". The mean ± SD for 3 animals is shown and all values are expressed in R.D.U. (relative density units) using β-actin as a reference. *, Significantly different versus chow diet-only group, $P<0.05$. **(B)** Representative Northern blots, from an independent experiment.

higher in males than in females. These results suggest that PPARγ activator troglitazone is effective in the treatment of dyslipidemia associated with metabolic syndrome because it is characterized by elevated plasma levels of triglycerides. In addition, it is likely that this effect of troglitazone is more prominent in males.

Steady state triglyceride levels are dependent on two pathways, endogenous synthesis and tissue clearance. Production of triglycerides in the liver is controlled in large part by substrate (fatty acids) availability, whereas tissue clearance of triglycerides is dependent on lipoprotein lipase activity and apo C-III (Dreyer et al., 1992; Staels et al., 1995; Peters et al., 1997). In our study, troglitazone affects both of these processes. Troglitazone was shown to decrease substrate availability due to up-regulated hepatic ACOX mRNA expression and to stimulate lipolysis and clearance of trigycerides due to down-regulated hepatic apo C-III mRNA expression, an apolipoprotein that limits tissue clearance of triglycerides. However, these activities of PPARγ on transcriptional expression of genes involved in triglyceride metabolism were much higher in males versus females. Moreover, mRNA levels of hepatic apo C-III were not changed by troglitazone treatment in female mice. Consistent with our data, the increase in ACOX with PPARγ agonist has also been reported in a comparable animal model. PPARγ agonist rosiglitazone induced enzymes of peroxisomal fatty acid β-oxidation, including ACOX in *db/db* mice (Edvardsson et al., 1999; Chaput et al., 2000). These results indicate that PPARγ may regulate hepatic ACOX and apo C-III gene expression and that differences in circulating triglyceride metabolism between two sexes are attributed in part to differences in these gene expression.

Reductions in serum triglycerides are felt to be the result of a specific coordinated shift in fatty acid flux from liver and muscle to adipose tissue which is linked to a PPARγ-mediated differentiation of preadipocytes, resulting in increased body weight (Tontonoz et al., 1995; Fruchart et al., 1998; Lupien et al., 1991; Yano et al., 1997). Pioglitazone and rosiglitazone have been shown to increase body weight gain in *db/db* mice and Zucker rats, while decreased serum triglycerides (Hirshman et al., 1995; Chaput et al., 2000). However, our present study showed that troglitazone decreased serum triglycerides without affecting the body weight gain in both sexes of C57BL/6J mice. According to the results from other investigators, troglitazone decreased serum triglycerides without changes in body weight gain and WAT mass in Sprague Dawley rats and male Zucker rats (Lefebvre et al., 1997; Okuno et al., 1998). They reported that troglitazone induced increases in small adipocytes without the change in white WAT mass and then may contribute to lower serum free fatty acid and triglyceride levels by TZDs. Our results also showed that WAT was not changed by troglitazone in both sexes of animals (data not shown). In general, troglitazone-activated PPARγ regulates genes involved in fatty acid β-oxidation and triglyceride metabolism and then decreases fatty acid and triglycerides responsible for adipocyte hypertrophy. However, troglitazone may not affect the body weight gain by increasing the number of small adipocytes.

In conclusion, the results of this study provide evidence that the PPARγ activator troglitazone reduces serum triglycerides with sexual dimorphism in C57BL/6J mice and that the actions of PPARγ on hepatic lipid metabolism may be influenced by sex-related factors. Further studies will be necessary to determine the factors contributing to these sex differences following troglitazone treatment and are needed to investigate its effects in adipose tissue as a major target tissue of PPARγ ligands, in order to be better understood the mechanisms that PPARγ regulates triglyceride metabolism.

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