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17β-estradiol Represses White Adipose Tissue Metabolism by Inhibiting PPARγ in High Fat Diet-induced Obese Female Ovariectomized Mice

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This study investigated whether increased adiposity is prevented by estrogen replacement in female ovariectomized (OVX) C57BL/6J mice, an animal model of human menopause and whether these metabolic changes reflect the inhibitory action of estrogen on peroxisome proliferator-activated receptor γ (PPARγ)-regulated gene expression. Treatment of 17β-estradiol for the last one week of the experiment decreased high fat diet-induced body weight gain and white adipose tissue mass compared to OVX control mice. Histological analysis showed that administration of 17β-estradiol to mice decreased the size of adipocytes in parametrial adipose tissue versus OVX control mice. In addition, 17β-estradiol reduced the adipose expression of PPARγ as well as PPARγ target genes such as adipocyte fatty acid binding protein and tumor necrosis factor α. These results suggest that 17β-estradiol may inhibit adiposity through reducing the PPARγ activities in female OVX mice.

Key Words: 17β-estradiol, PPARγ, White adipose tissue, Female mice

INTRODUCTION

Obesity arises from the imbalance between energy intake and energy expenditure, leading to a pathological accumulation of lipids in adipocytes. In white adipose tissue, preadipocytes exist in close proximity to adipocytes and respond to positive energy balance by proliferating and then differentiating into adipocytes (Kirkland et al., 1994). Mature adipocytes then accommodate excess energy through enhanced triacylglycerol storage.

Gonadal sex steroid hormone is known to regulate obesity and lipid metabolism (Mystkwski and Schwartz, 2000; Jeong and Yoon, 2007). The findings on estrogen regulation on fat deposition have generated considerable interest. Ovariectomy of experimental animals, or menopause in women resulting either from natural aging or surgical removal of the ovaries, results in increases in adipose tissue (Wade et al., 1985). ERα-knockout mice express more white adipose tissue compared with wild-type mice (Heine et al., 2000). Aromatase-knockout mice showed an increase in gonadal fat pad weight (Jones et al., 2000). The adipose changes in humans, rodents, or other animals caused by a lack of estrogen can be reversed with estrogen replacement (Mohamed and Abdel-Rahman, 2000), and decreases in adipose tissue are one significant benefit of hormone replacement therapy given to postmenopausal women (Harrbo et al., 1991). These models demonstrate the importance of estrogen signaling in adipose tissue physiology.

Energy balance is regulated by nuclear peroxisome proliferator-activated receptor (PPAR). Among PPAR isotypes, α, β/δ, and γ, the PPARγ is a major regulator of glucose and lipid metabolism by modulating energy homeostasis in adipose tissue (Semple et al., 2006). The activation of PPARγ is both necessary and sufficient to induce an adipose phenotype, which is defined by lipid accumulation and the expression of fat-specific marker genes such as adipocyte fatty acid binding protein (aP2), lipoprotein lipase, and adipsin (Rosen et al., 2000).

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It has been reported that PPARγ is capable of interacting with the estrogen receptor (ER) signal transduction pathway. The PPARγ/retinoid X receptor α (RXRα) complex binds to response elements (PPRE) in target genes. However, binding of PPAR/RXR inhibits transactivation by the ER through competitive binding (Keller et al., 1995). Then, we hypothesized that increased adiposity is prevented by estrogen replacement in the female ovariectomized (OVX) C57BL/6J mice, an animal model of postmenopausal women, and that these metabolic modulations reflect the inhibitory action of estrogen on the expression of PPARγ-regulated genes.

In present study, we examined whether estrogen regulates adipose tissue mass, adipocyte histology, and the expression of PPARγ and PPARγ target genes in adipose tissue of female OVX C57BL/6J mice.

MATERIALS AND METHODS

1. Animal treatments

For all experiments, eight-week-old female 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*. Female mice were each randomly divided into two groups. The first group was the high fat diet-fed OVX mice. The second group was the high fat diet-fed OVX mice subcutaneously implanted with 17β-estadiol (OVX + E, 0.05 mg/pellet, 60-day release) for the last one week of the experiment. All the animals received high fat diet (45% kcal fat, Research Diets, New Brunswick, NJ) for 6 weeks and were sacrificed by cervical dislocation. Tissues were harvested, weighed, snap frozen in liquid nitrogen and stored at -80℃ until use.

2. Histologic analysis and morphometry

Adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner for paraffin section. Sections (5 μm) were stained with hematoxylin and eosin for microscopic examination. For the quantitation of number and size of adipocytes, the sectional areas of adipose tissues in the hematoxylin and eosin-stained preparations were analyzed with image analysis system (Image pro-plus, MD, USA).

3. RNA prepartion and analysis

Total RNA was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY) and relative levels of specific mRNA were assessed by reverse transcription-polymerase chain reaction (RT-PCR). Complementary DNA was synthesized from RNA samples by mixing 2 μg of total RNA and 0.5 μg of the reverse primer in a total volume of 14 μl in water, heating the mixture at 75℃ for 15 min, cooling the mixture immediately on ice for 5 min, and adding 5× M-MLV reaction buffer, 10 mM dNTP mixture (Promega) and 200 units M-MLV RT (Promega) in total volume of 25 μl. Samples were incubated at 42℃ for 60 min. A five μl aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Twenty five μl PCR sample contained 5 μl of the RT reaction, 10× buffer with $MgCl_2$, 10 mM dNTP, 5 units of Tag polymerase (Solgent, Taejon, Korea) and 10 μM of each primer. Primer sequences and PCR conditions are

Table 1. Sequences of oligonucleotide primers and PCR conditions

Genes	Size (bp)	Primer sequences	Annealing (℃)	Cycle
PPARγ	340	Forward: 5'-attctggcccaccaacttcgg-3' Reverse: 5'-tggaagcctgatgctttatcccca-3'	58	28
aP2	417	Forward: 5'-caaaatgtgtgatgcctttgtg-3' Reverse: 5'-ctcttcctttggctcatgcc-3'	58	24
TNFα	387	Forward: 5'-ctcgagtgacaagcccgtag-3' Reverse: 5'-ttgacctcagcgctgagcag-3'	58	34
β-actin	350	Forward: 5'-tggaatcctgtggcatccatgaaa-3' Reverse: 5'-taaaacgcagctcagtaacagtcc-3'	58	28

shown in Table 1. PCR was performed in a PTC-100™ Programmable Thermal Controller (MJ Research, Watertown, MA, USA). PCR products were electophoresed on a 1% agarose gel.

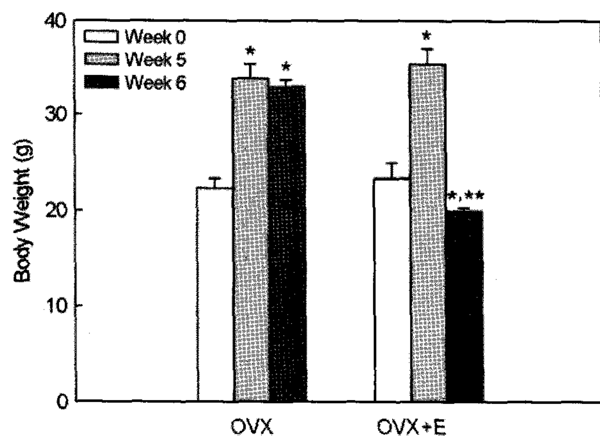


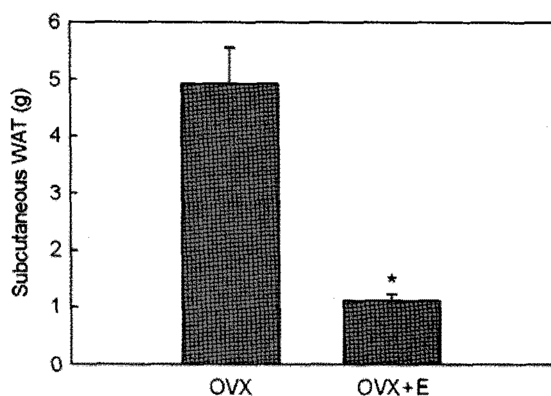
Fig. 1. Effects of 17β-estradiol on body weight in OVX female mice. C57BL/6J ovariectomized (OVX) mice (n=5/group) were treated with vehicle or 17β-estradiol (E). All values are expressed as mean ± SD. *P<0.05 Significantly different from week 0. **P<0.05 Significantly different from week 5.

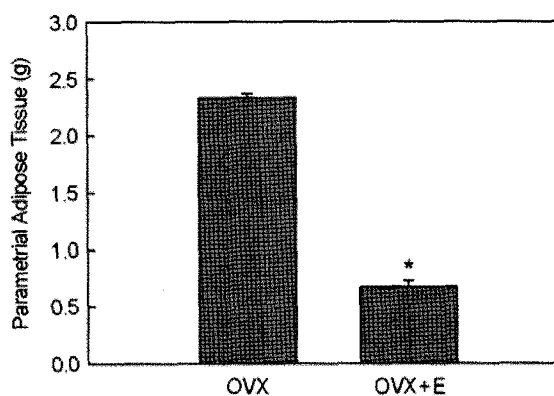
4. Statistics

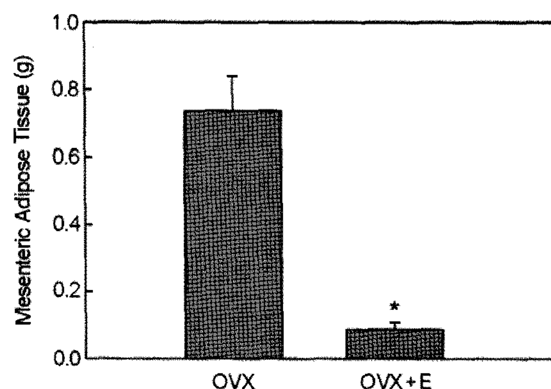
Unless otherwise noted, all values are expressed as mean ± standard deviation (SD). All data were analyzed by ANOVA for statistically significant differences between each group.

RESULTS

To determine whether 17β-estradiol regulates white adipose tissue metabolism in female OVX C57BL/6J mice, we preferentially measured body weight gain and adipose tissue mass. Compared to vehicle-treated OVX mice, treatment of 17β-estradiol for the last one week of experiment significantly decreased body weight (P<0.05) (Fig. 1). Similar to the results of body weight, 17β-estradiol treated







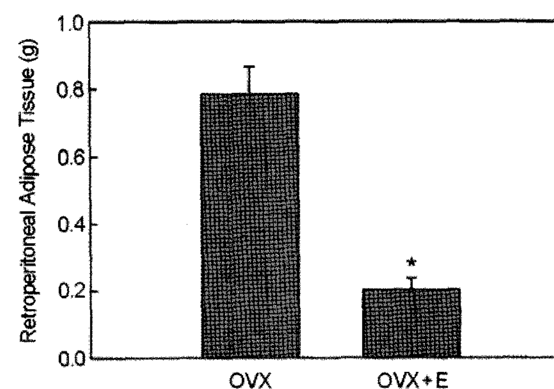


Fig. 2. Effects of 17β-estradiol on adipose tissue mass in OVX female mice. C57BL/6J ovariectomized (OVX) mice (n=5/group) were treated with vehicle or 17β-estradiol (E). All values are expressed as mean ± SD. *P<0.05 Significantly different from vehicle-treated OVX mice.

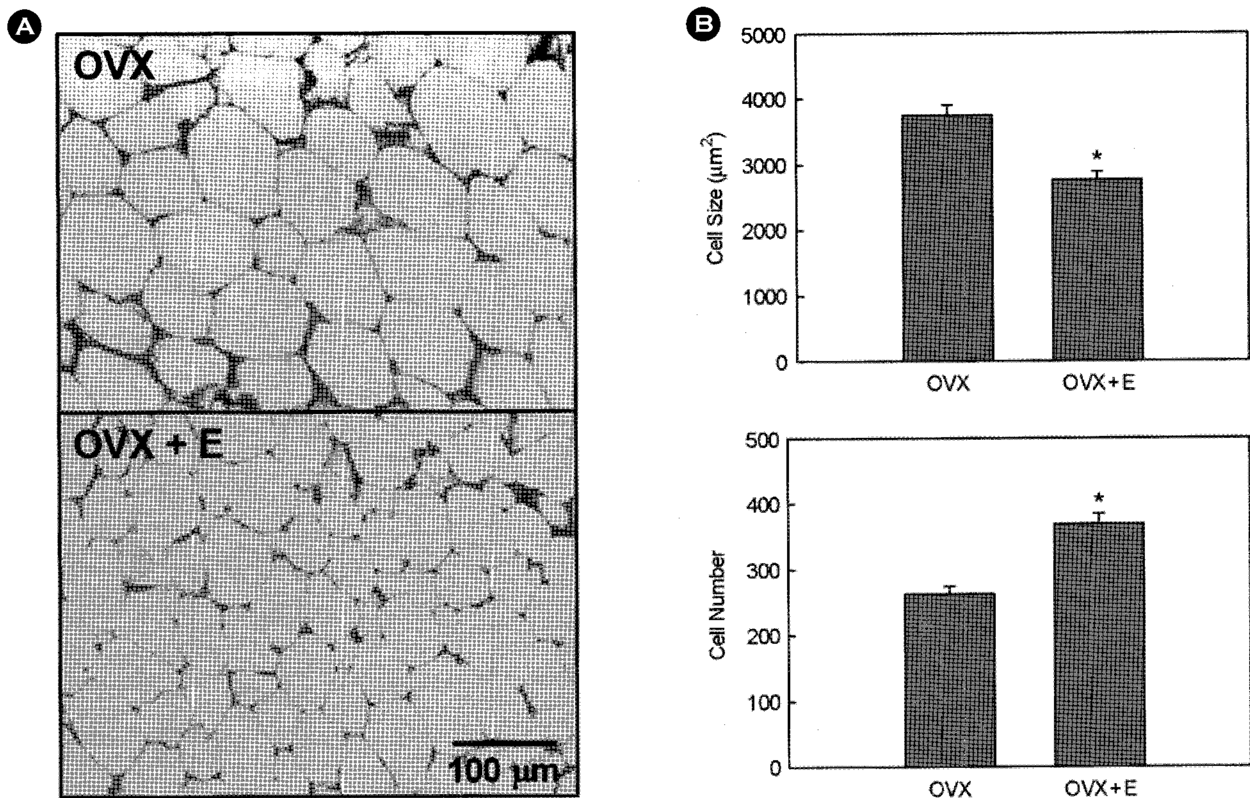


Fig. 3. Light microscopy of parametrial adipose tissue stained with hematoxylin and eosin (original magnification × 200). **(A)** C57BL/6J ovariectomized (OVX) mice (n=5/group) were treated with vehicle or 17β-estradiol (E). Shown are representative hematoxylin and eosin-stained sections (5 μm thick) of female parametrial adipose tissue. **(B)** Hematoxylin and eosin-stained sections were analyzed with an image analysis system, and the size and number of adipocytes in a fixed area (1,000,000 μm^2) were quantified. All values are expressed as mean ± SD. *$P<0.05$ Significantly different from vehicle-treated OVX mice.

mice had significantly decreased white adipose tissue weights compared with OVX mice ($P<0.05$) (Fig. 2).

Histological analysis showed that 17β-estradiol caused a 27% decrease in the size of adipocytes in parametrial white adipose tissue compared with OVX mice (Fig. 3). The number of adipocytes in a fixed area was increased by 40.5% in 17β-estradiol treated mice compared with OVX mice. Thus 17β-estradiol seems to stimulate the conversion of large adipocytes into small adipocytes in parametrial adipose tissue of female OVX mice, suggesting that 17β-estradiol regulates white adipose tissue metabolism and obesity in high fat diet fed female OVX mice.

To study whether the inhibitory effects of 17β-estradiol on body weight gain and white adipose tissue mass were caused by alteration of PPARγ activity in the white adipose tissue, we measured mRNA levels of the PPARγ (Fig. 4). Compared with the OVX mice, the 17β-estradiol treated OVX mice had substantially decreased levels of PPARγ mRNA by 42% in white adipose tissue.

We also tested the effects of 17β-estradiol on the expression of PPARγ target genes, such as adipocyte fatty acid binding protein (aP2) and tumor necrosis factor α (TNFα) mRNA in parametrial adipose tissue (Fig. 5). In parallel with reductions in PPARγ mRNA levels by 17β-estradiol, 17β-estradiol decreased mRNA expression of aP2 and TNFα. These results suggest that 17β-estradiol may down-regulate white adipose tissue metabolism through the inhibition of PPARγ activity.

DISCUSSION

In this study, we showed how 17β-estradiol can regulate white adipose tissue metabolism in high fat diet-induced obese female OVX mice. We also suggested here that PPARγ may be a molecular target for 17β-estradiol. 17β-estradiol decreased the mRNA expression of PPARγ and

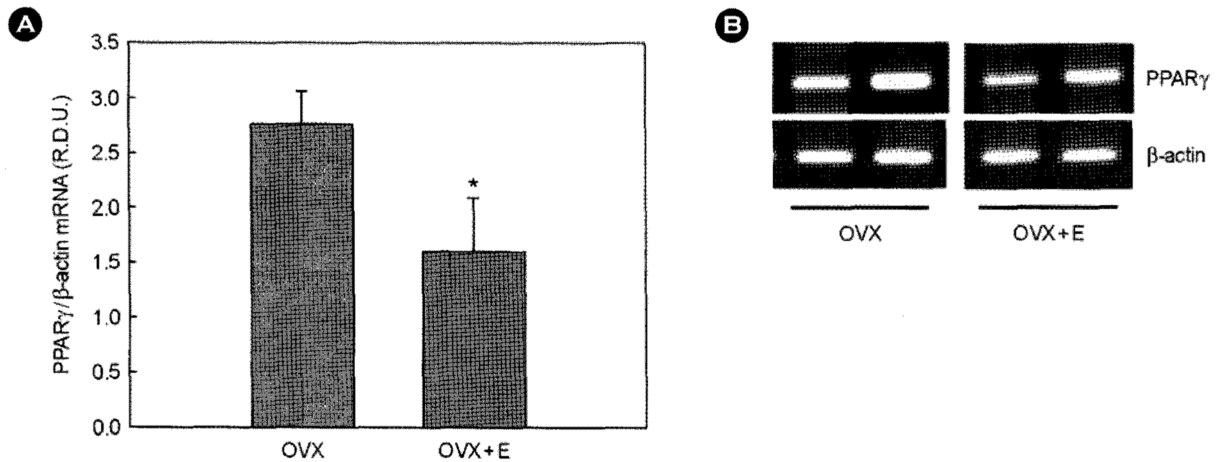


Fig. 4. Modulation of adipose PPARγ gene expression by 17β-estradiol in OVX female mice. **(A)** C57BL/6J ovariectomized (OVX) mice (n=5/group) were treated with vehicle or 17β-estradiol (E). RNA was extracted from the white adipose tissue, and all values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin. **(B)** Representative RT-PCR photographs from two of three independent experiments are shown. **P*<0.05 Significantly different from vehicle-treated OVX mice. RT-PCR; reverse transcription-polymerase chain reaction

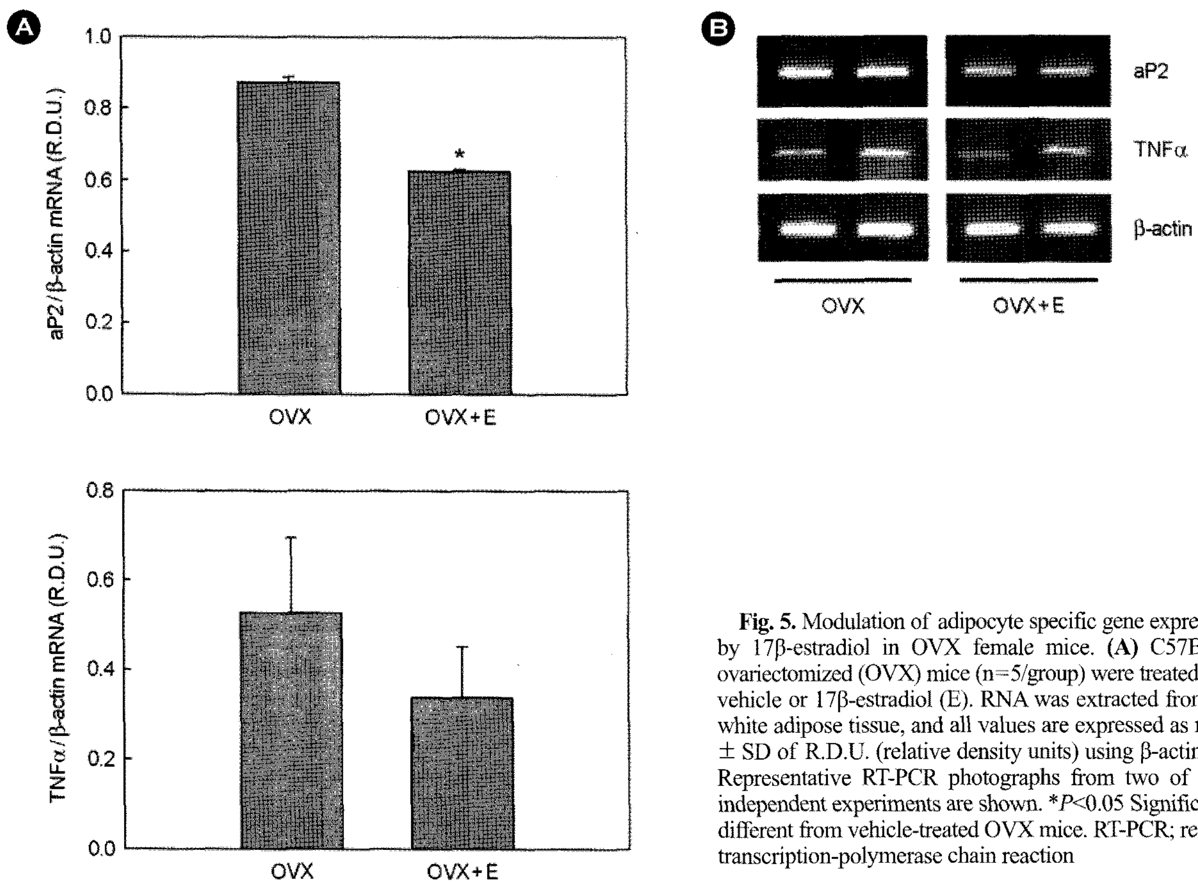


Fig. 5. Modulation of adipocyte specific gene expression by 17β-estradiol in OVX female mice. **(A)** C57BL/6J ovariectomized (OVX) mice (n=5/group) were treated with vehicle or 17β-estradiol (E). RNA was extracted from the white adipose tissue, and all values are expressed as mean ± SD of R.D.U. (relative density units) using β-actin. **(B)** Representative RT-PCR photographs from two of three independent experiments are shown. **P*<0.05 Significantly different from vehicle-treated OVX mice. RT-PCR; reverse transcription-polymerase chain reaction

PPARγ target genes, resulting in decreasing body weight gain and white adipose tissue mass in high fat diet-induced obese female OVX mice.

It has been known for many years that estrogen is an important regulator of female adipose deposition in humans, rodents, and other species (Wade et al., 1985). Ovariectomy of experimental animals, or menopause in women resulting either from natural aging or surgical removal of the ovaries,

results in increases in adipose tissue (Wade et al., 1985). Similarly, our results showed that body weight and white adipose tissue mass were increased by ovariectomy of female mice. However, 17β-estradiol treated OVX mice decreased body weight and white adipose tissue mass compared with OVX control mice. Thus, these results indicate that estrogen signaling produces a decrease in white adipose tissue mass.

With respect to morphological changes of adipocytes during obesity, 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). Since white adipose tissue lipids are largely derived from circulating triglycerides, adipocyte size seems to be influenced by serum trilgycerides (Bourgeois et al., 1983). 17β-estradiol has beneficial effects on lipid profiles and acts as efficient on lipid-lowering agents. In our previous report, 17β-estradiol treated OVX mice showed significant reductions in serum concentrations of triglycerides compare to high fat diet-fed OVX mice (Jeong and Yoon, 2007). This reports support our data that 17β-estradiol decreased the size of OVX-induced large adipocyte in parametrial adipose tissue. Thus our data indicate that 17β-estradiol may decrease the size of adipocytes in white adipose tissue of female OVX mice, resulting in decrease body weight and white adipose tissue weight.

To examine whether reductions in adipose tissue mass and adipocyte size by 17β-estradiol are associated with alterations in PPARγ activity in adipose tissue, we measured the mRNA expression of PPARγ and PPARγ target genes. 17β-estradiol treatment decreased the expression of PPARγ and PPARγ dependent adipoctye specific genes such as aP2 and TNFα compared with female OVX control mice. Reduced expression of PPARγ mRNA is expected to reduce lipogenesis, with concomitant reductions of adipocyte size. 17β-estradiol-dependent repression of adipose tissue aP2 expression would further inhibit lipid accumulation in adipocytes by decreasing the uptake of fatty acids from the circulation for triglycerides synthesis. It has reported that estrogen can modulate the adipogenic commitment of the mesenchymal mouse cell line KS483 via transcriptional repression of PPARγ (Dang et al., 2002). This report support our results that 17β-estradiol decrease lipogenesis of white adipose tissue through inhibition of PPARγ activity.

PPARγ modulates its target gene expression by binding to PPAR response elements (PPREs) after heterodimerization with RXR, playing a crucial role in adipogenesis and insulin sensitization (Camp et al., 2002; Rangwala and Lazar, 2004). An estrogen response element (ERE) is an inverted repeat containing three intervening bases (AGGTCA N_3 TGACCT), whereas a PPRE is a direct repeat with a single intervening sequences contain an AGGTCA half site (Klein-Hitpass et al., 1986; Tugwood et al., 1992). These sequences contain an AGGTCA half site, which could be recognized by either ER or PPAR. Signal cross-talk between PPAR/RXR and ER has been reported to occur through competitive binding to ERE (Keller et al., 1995). These reports support our data that 17β-estradiol inhibit PPARγ actions on white adipose tissue metabolism.

In conclusion, our results demonstrate that 17β-estradiol decreases adiposity and adipocyte size by reducing the expression of PPARγ target genes in adipose tissue of female OVX C57BL/6J mice.

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