

Signal crosstalk between estrogen and peroxisome proliferator-activated receptor α on adiposity

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Peroxisome proliferator-activated receptor α and estrogen are believed to be involved in metabolic changes leading to obesity. To test this relationship, we divided female wildtype and PPAR α -deficient mice fed on a high fat diet into the following groups: mock-operated, ovariectomized (OVX), and E₂-treated. The visceral white adipose tissue and plasma cholesterol levels were increased significantly in wild type OVX and decreased in the E₂-treated group, but interestingly not in PPAR α -deficient mice. The mRNA levels of lipoprotein lipase in adipose tissue were also increased in only wild type OVX and decreased significantly in E₂-treated mice. These novel results suggest the possibility of signaling crosstalk between PPAR α and E₂, causing obesity *in vivo*. [BMB reports 2009; 42(2): 91-95]

INTRODUCTION

Obesity is a disorder of energy balance, influenced by factors such as gender, nutrition, and genetics. Excess caloric intake induces the elevation of plasma triglycerides (TG) and cholesterol concentration. Elevated levels of TGs are stored in adipose tissue, resulting in hypertrophy and hyperplasia of adipocytes (1).

Estrogen, a steroid hormone, has a significant biological effect on adipose tissue. Female rodents that undergo ovariectomy become obese (2). Estrogen treatment reverses this condition, suggesting the involvement of estrogen in lipid metabolism in adipose tissue. Treatment of ovariectomized animals with estrogen decreased lipoprotein lipase (LPL) activity in adipose tissue (3, 4). LPL is postulated to reduce the release of free fatty acids, decrease TG assimilation, and diminish the size of adipocytes and body fat content.

Although several transcription factors can promote adipogenesis, their direct implication in mammalian obesity is not

fully substantiated. Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily, are one such example (5). PPARs form heterodimers with the retinoid X receptor (RXR) and bind to specific PPAR response elements in the promoter region of target genes (6). These play an important role in the regulation of genes involved in lipid utilization and storage, lipoprotein metabolism, adipocyte differentiation, and insulin action. PPAR α target genes include those involved in the hydrolysis of plasma TG; LPL and apolipoprotein CIII (apoCIII) (7), fatty acid uptake and binding; fatty acid transport protein and acyl-CoA synthetase (8), and fatty acid β -oxidation; acyl CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase (9). PPAR γ regulates genes involved in adipocyte differentiation, lipid storage, glucose metabolism, adipocyte fatty acid-binding protein, LPL, and leptin (10).

Lipid metabolism is affected by many hormonal signals, including classical hormones such as insulin, thyroid hormone, retinoic acid, glucocorticoids and estrogen, as well as other secreted molecules such as tumor necrosis factor- α and leptin. Previous *in vitro* reports demonstrated molecular mechanisms which involve crosstalk between the thyroid hormone receptor and PPAR through their common partner RXR (11), as well as between the estrogen receptor and PPAR α through their common binding to an estrogen response element (12). However, the correlation between PPAR α and estrogen on obesity is yet to be characterized *in vivo*.

In the present study, we investigated the correlation between PPAR α and estrogen using female wildtype and PPAR α -deficient mice, fed a high fat diet for 16 weeks. Furthermore, to elucidate a possible mechanism for the contribution of estrogen to adiposity, we studied the patterns of expression of enzymes related to lipid storage, LPL, PPAR γ , and leptin in white adipose tissue (WAT).

RESULTS AND DISCUSSION

Previous reports demonstrated that ovariectomized (OVX) female rodents became obese and that estrogen replacement reversed this condition (2, 13). In the present study, ovar-

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Table 1. Effect of estrogen (E₂) on body, uterine, and visceral WAT weight (Wt) in PPAR α ^{+/+} and PPAR α ^{-/-} mice

E ₂ Status	Surgery	n	Body weight (B.W.)		Uterine Wt (mg/g B.W.)	Visceral WAT Wt (g/g B.W.)
			Week 0	Week 16	Week 16	Week 16
PPARα^{+/+}						
Endogenous E ₂ (NOH)	Sham	7	21.45 ± 0.88	26.23 ± 1.86	6.13 ± 1.35	0.039 ± 0.008
E ₂ -deficient (OH)	OVX	7	23.43 ± 1.03	29.24 ± 1.99	1.20 ± 0.41	0.054 ± 0.008 ^a
Exogenous 17 β -E ₂ (OHE)	OVX+E ₂	7	21.73 ± 0.35	25.53 ± 1.40	13.93 ± 1.55	0.011 ± 0.000 ^b
PPARα^{-/-}						
Endogenous E ₂ (NOH)	Sham	11	16.40 ± 1.02	24.40 ± 1.40	4.30 ± 0.85	0.037 ± 0.009
E ₂ -deficient (OH)	OVX	8	20.20 ± 1.43	23.20 ± 0.64	1.16 ± 0.22	0.024 ± 0.011
Exogenous 17 β -E ₂ (OHE)	OVX+E ₂	9	21.40 ± 1.74	23.20 ± 1.82	13.33 ± 0.71	0.013 ± 0.006

Uterine weights were expressed in mg relative to body weight (mg/g B.W.) and visceral white adipose tissue (WAT) in g relative to body weight (g/g B.W.). n = number of mice examined. Results were expressed as mean ± standard deviation for all values.

^aP < 0.005 different from NOH group of PPAR α ^{+/+} mice.

^bP < 0.0005 different from OH group of PPAR α ^{+/+} mice.

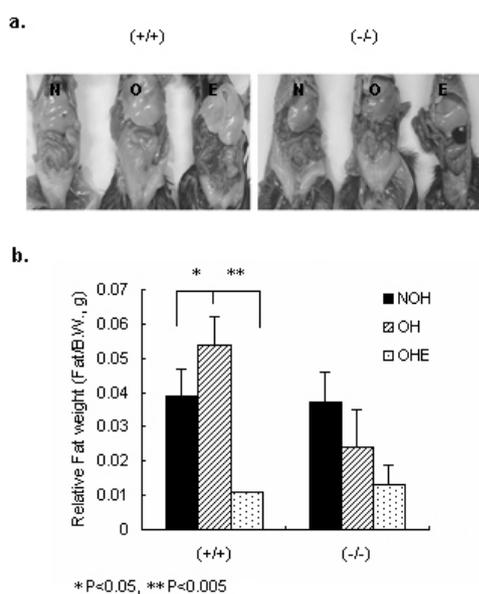


Fig. 1. Effect of estrogen on fat accumulation in PPAR α ^{+/+} and PPAR α ^{-/-} mice. (a) Gross finding of WAT in N, NOH; O, OH; E, OHE. (b) Visceral WAT weights relative to body weight (g of WAT/ g of body weight). NOH = non-ovariectomy, OH = ovariectomy, OHE = E₂-treated OH ovariectomy.

ovariectomy-atrophied uterus, as evidenced by uterine weight, did not cause significant changes in body weight between the different groups studied (Table 1). In PPAR α ^{+/+} mice, WAT was higher in the ovariectomized (OH) group than in the non-ovariectomized (NOH) group by 38% (P < 0.005). Furthermore, WAT was lower in the E₂-treated OH group (OHE) than in the OH group by 72% (P < 0.0005). However, PPAR α ^{-/-} mice did not display increased fat accumulation compared to the OH

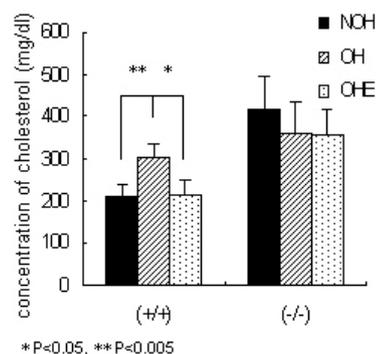


Fig. 2. Effect of estrogen on plasma cholesterol level in PPAR α ^{+/+} and PPAR α ^{-/-} mice. Plasma concentration of cholesterol (mg/dl) at week 16. NOH = non-ovariectomy, OH = ovariectomy, OHE = E₂-treated OH ovariectomy.

group. Instead, their WAT mass decreased by 35% compared to the NOH group (Table 1, Fig. 1).

E₂ has the ability to lower total plasma cholesterol both in animal models fed on a high fat diet and in women (14). Interestingly, the results of the present study showed that the changes in plasma cholesterol levels were similar to that of WAT weight, although all PPAR α ^{-/-} mice displayed higher basal levels of plasma cholesterol than PPAR α ^{+/+} mice, as previously reported (15). PPAR α ^{+/+} OVX mice showed an increase of 44% over the NOH group and a decrease of 28% following E₂ treatment, but PPAR α ^{-/-} mice did not (Fig. 2). There were no significant changes in the levels of TG, HDL, and LDL between the different genotypes studied (data not shown).

To determine the cause of these phenotypic differences between PPAR α ^{+/+} and PPAR α ^{-/-} mice, we investigated the expression of genes related to lipid storage and differentiation in the WAT of mice belonging to all experimental groups using

RT-PCR techniques. The levels of LPL mRNA expression, one of the important pathways by which estrogen suppresses fat accumulation, showed differential expression in the PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice. Similar to the observed change in WAT weight, PPAR $\alpha^{+/+}$ OVX mice demonstrated greater LPL mRNA expression than the PPAR $\alpha^{+/+}$ NOH group by 5% and the PPAR $\alpha^{+/+}$ OHE group exhibited lower LPL expression by 65% ($P < 0.005$). PPAR $\alpha^{-/-}$ OVX mice that did not gain WAT mass showed a 39% decrease rather than an increase ($P < 0.05$) in LPL mRNA expression (Fig. 3a).

The PPAR γ mRNA level showed a similar pattern of change in LPL expression between the different groups studied, al-

though this was not obvious. PPAR γ expression increased by 14% in the PPAR $\alpha^{+/+}$ OH group over the NOH group and decreased by 29% in the OHE group ($P < 0.05$). However, PPAR $\alpha^{-/-}$ mice showed a 20% and 36% decrease in the OH and OHE groups ($P < 0.05$), respectively (Fig. 3b). On the other hand, changes in leptin level between genotypes were not significantly different. All OVX mice showed a decrease in leptin expression over each NOH group regardless of genotype, namely 26% and 17% in PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice, respectively. Also, all E₂-treated mice showed an 85% and 51% decrease in PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice ($P < 0.05$), respectively (Fig. 3c). Collectively, we speculate that the difference between estrogen effects on obesity between PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ female mice might be related to the direct or indirect effect of estrogen on expression of LPL and PPAR γ mRNA levels.

The possibility of signal crosstalk between estrogen and PPAR has stimulated much interest because of their similar DNA target sequences (ERE, TGACCT N3 AGGTCA, PPRE, TGACCT N TCACCT), which Keller *et al.* (12) and Nunez *et al.* (16) reported based on *in vitro* studies. Due to the fact that estrogen and PPAR have a clinical impact on lipid metabolism, we hypothesized that crosstalk between the two factors may participate in the regulation of obesity *in vivo*. In the present study, we investigated the correlation of PPAR α and estrogen on adiposity by comparing OVX and E₂-treated PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice.

In PPAR $\alpha^{+/+}$ mice, ovariectomy induced increases in WAT mass and plasma cholesterol level, which were reversed by E₂ treatment. Such findings agree with those of previous reports, which concluded that estrogen decreased total serum cholesterol (17) and fat deposition, especially in visceral adipose tissues (18). However, the increases in WAT and plasma cholesterol level following ovariectomy were not observed in PPAR $\alpha^{-/-}$ mice, suggesting that PPAR α and estrogen might act together to regulate obesity.

Concurrent with the changes in WAT and plasma cholesterol levels between the different genotypes, PPAR $\alpha^{+/+}$ OVX mice showed increased mRNA levels of LPL, an enzyme that is down-regulated by estrogen, and PPAR γ , a transcription factor that increases LPL in adipose tissue. The mRNA levels of both genes were decreased in the OHE group. The observed change in LPL level according to E₂ status was consistent with other reports (3, 4). Interestingly, however, the mRNA levels of those genes in PPAR $\alpha^{-/-}$ mice were not increased in the OH group. This inferred that the difference in effect exerted by E₂ between the PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice may be due to the direct or indirect action of LPL and PPAR γ . LPL is a PPRE target gene, based on the identification of a PPRE in the LPL promoter (5). Selective PPAR α and PPAR γ ligands increase LPL expression in the liver and adipose tissue, respectively (19). However, Ranganathan and Kern (20) recently reported that direct PPAR γ activation actually reduced LPL activity in cultured adipocytes by a posttranslational inhibitory mechanism. Therefore, PPAR

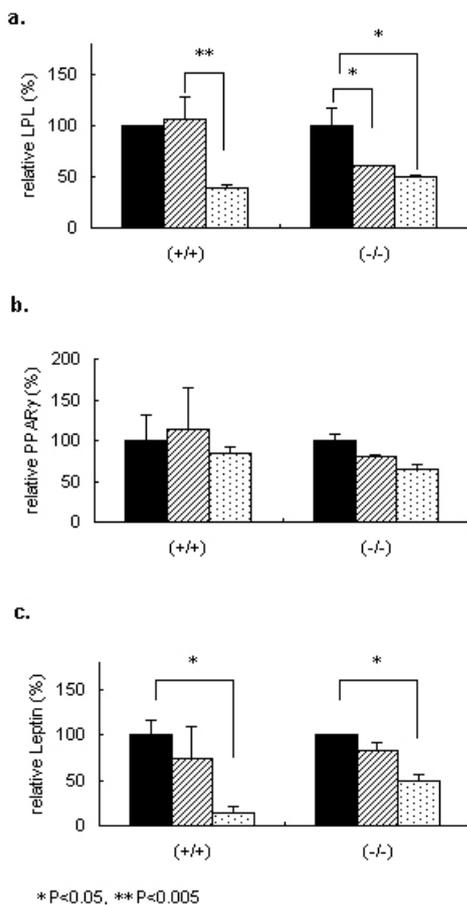


Fig. 3. RT-PCR analyses of LPL, PPAR γ , and leptin expression in WAT in PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice. NOH = non-ovariectomy, OH = ovariectomy, OHE = E₂-treated OH ovariectomy. Data represent mean and standard deviation of at least three independent experiments. Vertical bars represent the level of mRNA of different genes. Each value was normalized to β -actin (a) were expressed again as values (b) relative to each normalized gene expression of the NOH group ($b = a / \text{values of PPAR}\alpha^{+/+} \text{ or PPAR}\alpha^{-/-} \text{ NOH} \times 100$). Black bar = NOH, hatched bar = OH, dotted bar = OHE.

regulation of LPL activity involves a complex interplay of different mechanisms.

Our study also supported the complex interplay of PPAR in the regulation of LPL activity by showing different results in regulating adiposity between PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice. Furthermore, estrogen showed a negative regulatory activity on the LPL promoter through putative activating protein-1 (AP-1 protein(s)) (21), and PPARs interfere with the AP-1 and nuclear factor- κ B (NF- κ B) signaling pathway by preventing the binding of AP-1 and NF- κ B proteins to their target sequences (22). Therefore, it was inferred that a certain unknown signal crosstalk between PPAR α and estrogen might be responsible for the different results of LPL activity between PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice in this study.

On the other hand, the degree of mRNA expression level of leptin was similar between genotypes and was not affected by estrogen in either the PPAR $\alpha^{+/+}$ or PPAR $\alpha^{-/-}$ group. Expression of leptin can be increased by overfeeding as part of a feedback mechanism to limit further food intake and weight gain (23). Because PPAR $\alpha^{-/-}$ mice are not hyperphagic (24, 25) the genotype did not affect leptin levels. Also, the leptin level was not significantly altered by estrogen because estrogen did not directly regulate leptin secretion or its effects on fat mass (26). This supported the finding that the expression of leptin did not significantly affect differential fat accumulation between the PPAR α genotypes.

In conclusion, our data suggests that estrogen-controlled adiposity depends on PPAR α , and that LPL and PPAR γ might be involved in the mechanism. These results are the first to suggest the possibility of signal crosstalk between PPAR α and estrogen on adiposity *in vivo*. However, further study is required to uncover the exact mechanism causing the different effects of estrogen between PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice, and to understand the activity of PPAR α in obesity and lipid metabolism in females.

MATERIALS AND METHODS

Animals and treatment

Specific pathogen-free C57BL/6N \times 129/Sv homozygous PPAR α wildtype (+/+) and deficient (-/-) mice were initially introduced from the National Institute of Health (Bethesda, MD, USA) and bred at the Korea Research Institute of Bioscience and Biotechnology. Female PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice were treated similarly as follows: at sexual maturity (5 weeks old), female mice were bilaterally ovariectomized (OVX) or underwent a mock operation after anesthetization with avertin (0.02 μ g/g). Two weeks after surgery, some of the OVX mice were implanted with a subcutaneous slow-release hormone pellet (Innovative Research of America, Sarasota, Florida, USA) twice for 16 weeks (at day 1 and day 61). The pellets were designed to release 12 μ g/day exogenous E $_2$ as 17 β -estradiol for 60 days. The animals were fed on a high fat diet containing 15% fat, 1.25% cholesterol, 0.5% Na-cholate (Oriental Yeast CO, Ltd,

Japan) for 16 weeks. Their body weights were measured weekly and blood was collected at week one, four, eight, and sixteen following initiation of the high fat diet. After 16 weeks, the animals were sacrificed by cervical dislocation, and the liver and WAT were excised, weighed, snap frozen in liquid nitrogen, and stored at -70 $^{\circ}$ C until further use. The entire uterus was collected from each mouse to assess *in vivo* exposure to E $_2$. Serosal fat was removed with a pair of fine scissors. After removal of luminal fluid, the entire uterus was wet-weighed. The Ethics Committee of Korea Research Institute of Bioscience and Biotechnology approved of all animal use.

Blood chemical analyses

Blood was collected from the retro-orbital venous plexus with heparinized capillary tubes. Plasma was obtained by centrifugation of the whole blood at 10,786 g at 4 $^{\circ}$ C for 10 min and stored at -70 $^{\circ}$ C before further analysis. Plasma total cholesterol, TG, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured using an automatic blood chemical analyzer (7020, Hitachi, Japan).

Reverse transcriptase-polymerase chain reaction analysis

Total RNA was isolated from WAT of all mice using TRIZOL (Gibco BRL, Rockville, MD, USA) according to the manufacturer's recommended procedure. cDNA was synthesized from 1 μ g of total RNA using moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA). 2 μ l of synthesized total cDNA were used for PCR. The sense and anti-sense primer sequences were: 5'-CAGCAAGACCTTCGTGGTG A-3' and 3'-GTACAGGGCGGCCACAAGT-5' for LPL (84 bp product); 5'-TGACTCATAATAAGTC-3' and 3'-CATCTAATT CCAGTGCAT-5' for PPAR γ (485 bp product); 5'-GTGCTGGA GACCCCTGTGTCG-3' and 3'-AGAATGGGGTGAAGCCCAG GA-5' for leptin (207 bp product); and 5'-TGGAATCCTGTGG CATCCATGAAAC-3' and 3'-TAAACGCAGCTCAGTAACAGT CCG-5' for β -actin (349-bp product). The amplification products were separated electrophoretically on a 1.5% agarose gel containing ethidium bromide. UV-stimulated fluorescence was captured using a digital videocamera and quantitated with the Bio 1D software (Vilber Lourmat, Marine, Cedex, France). Linearity of the PCR was tested by amplifying each cDNA at various numbers of cycles and was found to be between 25 and 35 cycles. All experimental values were normalized to β -actin.

Statistics

Data were presented as the mean \pm standard deviation and statistical significance was determined by the Student's t-test. Differences were considered significant when P was less than 0.05.

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