BMB
reports

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

Bang Hyun Kim[1], Young Suk Won[2], Dae Yong Kim[3], Bora Kim[5], Eun-Young Kim[5], Mijung Yoon[4] & Goo Taeg Oh[5,]*

[1]Department of Toxicology, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, [2]Bio-Evaluation Center, KRIBB, Cheongwon, Chungbuk 363-883, [3]Department of Veterinary Pathology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, [4]Department of Life Science, Mokwon University, Daejeon 302-729, [5]Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Korea

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 PPARα-deficient mice fed on a high fat diet into the following groups: mock-operated, ovariectomized (OVX), and E_2-treated. The visceral white adipose tissue and plasma cholesterol levels were increased significantly in wild type OVX and decreased in the E_2-treated group, but interestingly not in PPARα-deficient mice. The mRNA levels of lipoprotein lipase in adipose tissue were also increased in only wild type OVX and decreased significantly in E_2-treated mice. These novel results suggest the possibility of signaling crosstalk between PPARα and E_2, 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. PPARα 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). PPARγ 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 PPARα through their common binding to an estrogen response element (12). However, the correlation between PPARα and estrogen on obesity is yet to be characterized *in vivo*.

In the present study, we investigated the correlation between PPARα and estrogen using female wildtype and PPARα-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, PPARγ, 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-

*Corresponding author. Tel: 82-2-3277-4128; Fax: 82-2-3277-3760; E-mail: gootaeg@ewha.ac.kr

Received 9 December 2008, Accepted 11 December 2008

Keywords: Estrogen, LPL, Obesity, PPARα, Signaling crosstalk

Table 1. Effect of estrogen (E_2) on body, uterine, and visceral WAT weight (Wt) in PPARα$^{+/+}$ and PPARα$^{-/-}$ 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 17β-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.
[a]$P < 0.005$ different from NOH group of PPARα$^{+/+}$ mice.
[b]$P < 0.0005$ different from OH group of PPARα$^{+/+}$ mice.

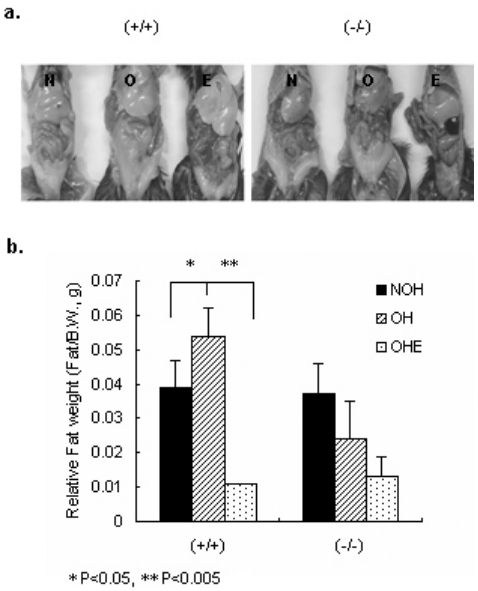

Fig. 1. Effect of estrogen on fat accumulation in PPARα$^{+/+}$ and PPARα$^{-/-}$ mice. (a) Gross finding of WAT in N, NOH; O, OH; E, OHE. (b) Visceral WAT weights relative to body weight (g of WAT/ g of body weight). NOH = non-ovariectomy, OH = ovariectomy, OHE = E_2-treated OH ovariectomy.

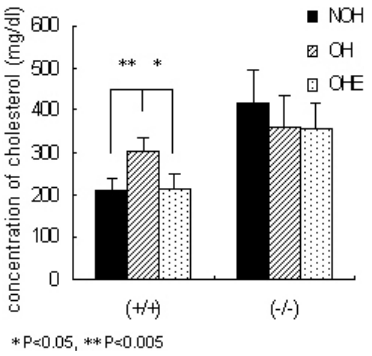

Fig. 2. Effect of estrogen on plasma cholesterol level in PPARα$^{+/+}$ and PPARα$^{-/-}$ mice. Plasma concentration of cholesterol (mg/dl) at week 16. NOH = non-ovariectomy, OH = ovariectomy, OHE = E_2-treated OH ovariectomy.

iectomy-atrophied uterus, as evidenced by uterine weight, did not cause significant changes in body weight between the different groups studied (Table 1). In PPARα$^{+/+}$ 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 E_2-treated OH group (OHE) than in the OH group by 72% ($P < 0.0005$). However, PPARα$^{-/-}$ mice did not display increased fat accumulation compared to the OH group. Instead, their WAT mass decreased by 35% compared to the NOH group (Table 1, Fig. 1).

E_2 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 PPARα$^{-/-}$ mice displayed higher basal levels of plasma cholesterol than PPARα$^{+/+}$ mice, as previous reported (15). PPARα$^{+/+}$ OVX mice showed an increase of 44% over the NOH group and a decrease of 28% following E_2 treatment, but PPARα$^{-/-}$ 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 PPARα$^{+/+}$ and PPARα$^{-/-}$ 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 PPARα$^{+/+}$ and PPARα$^{-/-}$ mice. Similar to the observed change in WAT weight, PPARα$^{+/+}$ OVX mice demonstrated greater LPL mRNA expression than the PPARα$^{+/+}$ NOH group by 5% and the PPARα$^{+/+}$ OHE group exhibited lower LPL expression by 65% ($P < 0.005$). PPARα$^{-/-}$ 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 PPARγ mRNA level showed a similar pattern of change in LPL expression between the different groups studied, although this was not obvious. PPARγ expression increased by 14% in the PPARα$^{+/+}$ OH group over the NOH group and decreased by 29% in the OHE group ($P < 0.05$). However, PPARα$^{-/-}$ 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 PPARα$^{+/+}$ and PPARα$^{-/-}$ mice, respectively. Also, all E_2-treated mice showed an 85% and 51% decrease in PPARα$^{+/+}$ and PPARα$^{-/-}$ mice ($P < 0.05$), respectively (Fig. 3c). Collectively, we speculate that the difference between estrogen effects on obesity between PPARα$^{+/+}$ and PPARα$^{-/-}$ female mice might be related to the direct or indirect effect of estrogen on expression of LPL and PPARγ mRNA levels.

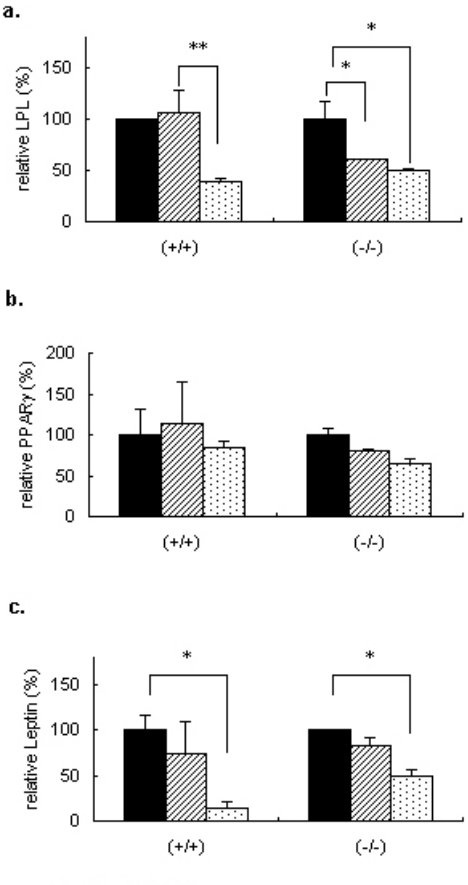

Fig. 3. RT-PCR analyses of LPL, PPARγ, and leptin expression in WAT in PPARα$^{+/+}$ and PPARα$^{-/-}$ mice. NOH = non-ovariectomy, OH = ovariectomy, OHE = E_2-treated OH ovariectomy. Data represent mean and standard deviation of at least three independent experiments. Vertical bars represent the level 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/values of PPARα$^{+/+}$ or PPARα$^{-/-}$ NOH ×100). Black bar = NOH, hatched bar = OH, dotted bar = OHE.

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 PPARα and estrogen on adiposity by comparing OVX and E_2-treated PPARα$^{+/+}$ and PPARα$^{-/-}$ mice.

In PPARα$^{+/+}$ mice, ovariectomy induced increases in WAT mass and plasma cholesterol level, which were reversed by E_2 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 PPARα$^{-/-}$ mice, suggesting that PPARα and estrogen might act together to regulate obesity.

Concurrent with the changes in WAT and plasma cholesterol levels between the different genotypes, PPARα$^{+/+}$ OVX mice showed increased mRNA levels of LPL, an enzyme that is down-regulated by estrogen, and PPARγ, 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 E_2 status was consistent with other reports (3, 4). Interestingly, however, the mRNA levels of those genes in PPARα$^{-/-}$ mice were not increased in the OH group. This inferred that the difference in effect exerted by E_2 between the PPARα$^{+/+}$ and PPARα$^{-/-}$ mice may be due to the direct or indirect action of LPL and PPARγ. *LPL* is a PPAR target gene, based on the identification of a PPRE in the LPL promoter (5). Selective PPARα and PPARγ ligands increase LPL expression in the liver and adipose tissue, respectively (19). However, Ranganathan and Kern (20) recently reported that direct PPARγ activation actually reduced LPL activity in cultured adipocytes by a posttranslational inhibitory mechanism. Therefore, PPAR

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 PPARα$^{+/+}$ and PPARα$^{-/-}$ 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-κB (NF-κB) signaling pathway by preventing the binding of AP-1 and NF-κB proteins to their target sequences (22). Therefore, it was inferred that a certain unknown signal crosstalk between PPARα and estrogen might be responsible for the different results of LPL activity between PPARα$^{+/+}$ and PPARα$^{-/-}$ 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 PPARα$^{+/+}$ or PPARα$^{-/-}$ 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 PPARα$^{-/-}$ 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 PPARα genotypes.

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

MATERIALS AND METHODS

Animals and treatment

Specific pathogen-free C57BL/6N×129/Sv homozygous PPARα 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 PPARα$^{+/+}$ and PPARα$^{-/-}$ 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 μg/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 μg/day exogenous E_2 as 17β-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°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°C for 10 min and stored at -70°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 μg of total RNA using moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA). 2 μl of synthesized total cDNA were used for PCR. The sense and antisense primer sequences were: 5'-CAGCAAGACCTTCGTGGTG A-3' and 3'-GTACAGGGCGGCCACAAGT-5' for LPL (84 bp product); 5'-TGACTCATACATAAAGTC-3' and 3'-CATCTAATT CCAGTGCAT-5' for PPARγ (485 bp product); 5'-GTGCTGGA GACCCCTGTGTCG-3' and 3'-AGAATGGGGTGAAGCCCAG GA-5' for leptin (207 bp product); and 5'-TGGAATCCTGTGG CATCCATGAAAC-3' and 3'-TAAAACGCAGCTCAGTAACAGT 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 ± standard deviation and statistical significance was determined by the Student's t-test. Differences were considered significant when P was less than 0.05.

Acknowledgements

This work was supported by a grant from the Korean Ministry of Heath and Welfare (A000385) and a grant from the Seoul R & BD Program (11117M0214882).

REFERENCES

1. Bourgeois, F., Alexiu, A. and Lemonnier, D. (1983) Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice. *Br. J. Nutr.* **49**, 17-26.
2. Pedersen, S.B., Borglum, J.D., Moller-Pedersen, T. and Richelsen, B. (1992) Effects of *in vivo* estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. *Mol. Cell Endocrinol.* **85**, 13-19.
3. Iverius, P.H. and Brunzell, J.D. (1988) Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. *J. Clin. Invest.* **82**, 1106-1112.
4. Peinado-Onsurbe, J., Staels, B., Vanderschueren, D., Bouillon, R. and Auwerx, J. (1993) Effects of sex steroids on hepatic and lipoprotein lipase activity and mRNA in the rat. *Horm. Res.* **40**, 184-188.
5. Schoonjans, K., Staels, B. and Auwerx, J. (1996) The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim. Biophys. Acta.* **1302**, 93-109.
6. Desvergne, B. and Wahli, W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20**, 649-688.
7. Auwerx, J., Schoonjans, K., Fruchart, J.C. and Staels, B. (1996) Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis* **124**, S29-37.
8. Martin, G., Schoonjans, K., Lefebvre, A.M., Staels, B. and Auwerx, J. (1997) Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J. Biol. Chem.* **272**, 28210-28217.
9. Fan, C.Y., Pan, J., Usuda, N., Yeldandi, A.V., Rao, M.S. and Reddy, J.K. (1998) Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. *J. Biol. Chem.* **273**, 15639-15645.
10. Spiegelman, B.M. (1998) PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* **47**, 507-514.
11. Juge-Aubry, C.E., Gorla-Bajszczak, A., Pernin, A., Lemberger, T., Wahli, W., Burger, A.G. and Meier, C.A. (1995) Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. *J. Biol. Chem.* **270**, 18117-18122.
12. Keller, H., Givel, F., Perroud, M. and Wahli, W. (1995) Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. *Mol. Endocrinol.* **9**, 794-804.
13. Pedersen, S.B., Borglum, J.D., Eriksen, E.F. and Richelsen, B. (1991) Nuclear estradiol binding in rat adipocytes. Regional variations and regulatory influences of hormones. *Biochim. Biophys. Acta.* **1093**, 80-86.
14. Nathan, L. and Chaudhuri, G. (1997) Estrogens and atherosclerosis. *Annu. Rev. Pharmacol. Toxicol.* **37**, 477-515.
15. Peters, J.M., Hennuyer, N., Staels, B., Fruchart, J.C., Fievet, C., Gonzalez, F.J. and Auwerx, J. (1997) Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J. Biol. Chem.* **272**, 27307-27312.
16. Nunez, S.B., Medin, J.A., Braissant, O., Kemp, L., Wahli, W., Ozato, K. and Segars, J.H. (1997) Retinoid X receptor and peroxisome proliferator-activated receptor activate an estrogen responsive gene independent of the estrogen receptor. *Mol. Cell. Endocrinol.* **127**, 27-40.
17. Stampfer, M.J., Colditz, G.A., Willett, W.C., Manson, J.E., Rosner, B., Speizer, F.E. and Hennekens, C.H. (1991) Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. *N. Engl. J. Med.* **325**, 756-762.
18. Haarbo, J., Marslew, U., Gotfredsen, A. and Christiansen, C. (1991) Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. *Metabolism* **40**, 1323-1326.
19. Lefebvre, A.M., Peinado-Onsurbe, J., Leitersdorf, I., Briggs, M.R., Paterniti, J.R., Fruchart, J.C., Fievet, C., Auwerx, J. and Staels, B. (1997) Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler. *Thromb. Vasc. Biol.* **17**, 1756-1764.
20. Ranganathan, S. and Kern, P.A. (1998) Thiazolidinediones inhibit lipoprotein lipase activity in adipocytes. *J. Biol. Chem.* **273**, 26117-26122.
21. Homma, H., Kurachi, H., Nishio, Y., Takeda, T., Yamamoto, T., Adachi, K., Morishige, K., Ohmichi, M., Matsuzawa, Y. and Murata, Y. (2000) Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *J. Biol. Chem.* **275**, 11404-11411.
22. Delerive, P., Martin-Nizard, F., Chinetti, G., Trottein, F., Fruchart, J.C., Najib, J., Duriez, P. and Staels, B. (1999) Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. *Circ. Res.* **85**, 394-402.
23. Kersten, S., Desvergne, B. and Wahli, W. (2000) Roles of PPARs in health and disease. *Nature* **405**, 421-424.
24. Costet, P., Legendre, C., More, J., Edgar, A., Galtier, P. and Pineau, T. (1998) Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273**, 29577-29585.
25. Guerre-Millo, M., Gervois, P., Raspe, E., Madsen, L., Poulain, P., Derudas, B., Herbert, J.M., Winegar, D.A., Willson, T.M., Fruchart, J.C., Berge, R.K. and Staels, B. (2000) Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J. Biol. Chem.* **275**, 16638-16642.
26. Pelleymounter, M.A., Baker, M.B. and McCaleb, M. (1999) Does estradiol mediate leptin's effects on adiposity and body weight? *Am. J. Physiol.* **276**, E955-963.