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Morphological Changes in Adipose and Liver Tissues by 17β-estradiol in Female Ovariectomized C57BL/6J Mice

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To determine whether 17β-estradiol induces the morphological changes in adipose and liver tissues, we measured the effects of 17β-estradiol on adipose tissue mass, adipocyte histology and hepatic lipid accumulation in female ovariectomized (OVX) C57BL/6J mice. Compared to vehicle-treated control mice, 17β-estradiol-treated mice decreased adipose tissue mass and the size of adipocytes, and concomitantly increased the number of adipocytes in a dose-dependent manner. In addition, the administration of 17β-estradiol resulted in reduced hepatic lipid accumulation in a dose-dependent manner. These results suggest that estrogen may regulate adipocyte development and lipid metabolism in female OVX C57BL/6J mice.

Key Words: Estrogen, Adipocyte development, Lipid metabolism, OVX female

INTRODUCTION

Obesity causes major health problems. It is a risk factor for non-insulin-dependent diabetes, cardiovascular disease, osteoarthritis, some types of cancers, and certain reproductive and metabolic disorders (Bray, 2003). This risk generally relates to the distribution and amount of adipose tissue.

Sex steroid hormones are involved in the metabolism, accumulation and distribution of adipose tissues (Mystkwski et al., 2000). Gonadal steroids have been the subject of intense investigation over the last several decades because of the role that these ovarian hormones have in the regulation of food intake, body weight and lipid metabolism. For example, it is well known that ovariectomized (OVX) animals and postmenopausal women show increased food intake, body weight, and adipose tissue mass, indicating the involvement of gonadal steroids in the modulation of obesity (Czaja et al., 1983; Garcia Rodriguez et al., 1990; Geary et al., 2001; Wade 1975; Wing et al., 1991). With a decrease in sex steroid hormones as a result of aging or gonadectomy, there is a tendency to increase adipose tissue mass (Bjorntorp, 1996). In fact, hormone replacement therapy in postmenopausal women appears to reduce the degree of adipose tissue mass (Wade et al., 1979; Tchernof et al., 1998). In addition, ovarian steroids can affect adipose tissue mass and lipid metabolism, the effects of which are probably mediated by estrogen (Mystkwski et al., 2000). Estrogen insufficiency is known to be largely responsible for increased adiposity and circulating lipids in OVX rodents, because such animals do not display obesity, adiposity and lipid disorders when they are administered estrogen (Shearer et al., 2000; Shinoda et al., 2002). Thus this suggests that estrogen modulates adipocyte development and lipid metabolism.

Therefore, the objective of the present study was to determine whether the most potent estrogen, 17β-estradiol regulates morphological changes in adipose and liver tissues of female OVX C57BL/6J mice. Here we report that 17β-estradiol affects adipose tissue weight, adipocyte size and number as well as hepatic lipid accumulation in a dose-dependent manner.

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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 special administration, mice were fed standard rodent chow and water *ad libitum*. Mice were OVX, each randomly divided into 4 groups, and received once daily intraperitoneal injections of 17β-estadiol (Sigma) at the indicated doses for 8 days. 17β-estadiol was dissolved in corn oil and chow diet-fed control mice were treated with corn oil-only. All the animals received a regular chow fat diet (4.5% fat w/w, CJ Corp., Korea) 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 and liver 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

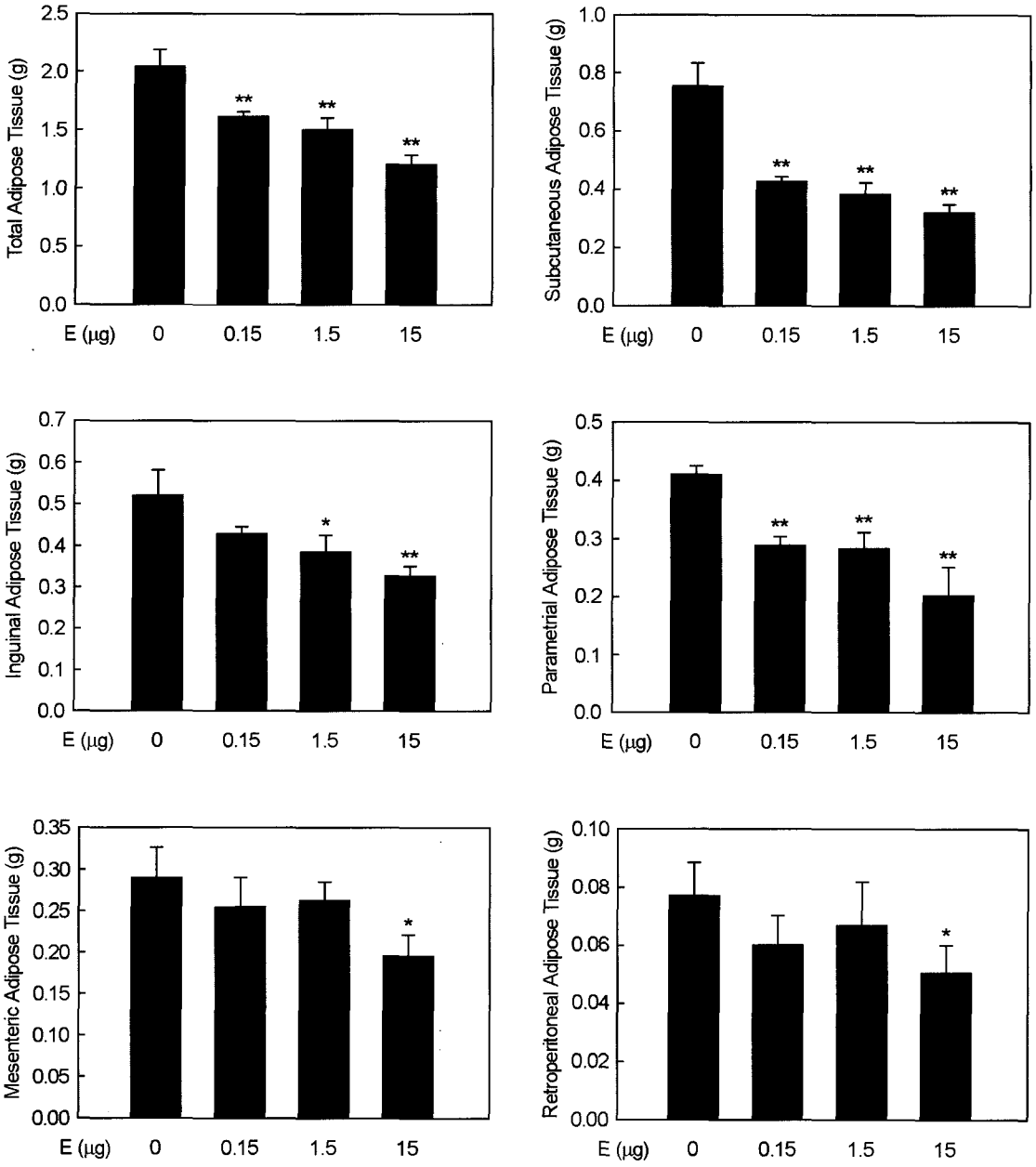

Fig. 1. Effects of 17β-estradiol on adipose tissue mass in female OVX mice. Mice received intraperitoneal injections of 17β-estradiol at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. All values are expressed as mean ± SD. *, Significantly different *versus* control group ($P<0.05$). **, Significantly different *versus* control group ($P<0.01$).

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 an image analysis system (Image pro-plus, MD, USA).

3. 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 the effects of pharmacological doses of 17β-estradiol on adipose tissue mass, female OVX C57BL/6J mice were treated once daily with 17β-estradiol for 8 days. Compared with vehicle-treated control mice, mice treated with 17β-estradiol significantly reduced adipose tissue mass in a dose-dependent manner and the maximal decrease of adipose tissue mass was achieved at dose of 15 μg per mouse for 17β-estradiol ($P<0.01$) (Fig. 1).

We determined whether 17β-estradiol regulates adipocyte size and number in female OVX mice. Compared with control mice, mice treated with 17β-estradiol for 8 days significantly reduced adipocyte size and increased adipocyte number in a dose-dependent manner (Figs. 2 and 3). The treatment of 17β-estradiol (15 μg per mouse) for 8 days ($P<0.005$) resulted in a 48% decrease of adipocyte size and a 92% increase of adipocyte number.

Since WAT lipids are largely derived from circulating triglycerides, adipocyte size seems to be influenced by serum triglycerides (Bourgeois et al., 1983). To determine whether the changes in adipocyte size and number are due to reduced liver lipids that regulate serum lipid metabolism, we determined hepatic lipid accumulation (Fig. 4). The hepatic accumulation of lipids was significantly inhibited by 17β-estradiol treatment compared with chow-fed control mice. Similar to the effects of 17β-estradiol on adipose tissue mass, mice treated with 17β-estradiol (15 μg per mouse) for 8 days showed the highest inhibition of hepatic lipid accumulation (Fig. 4).

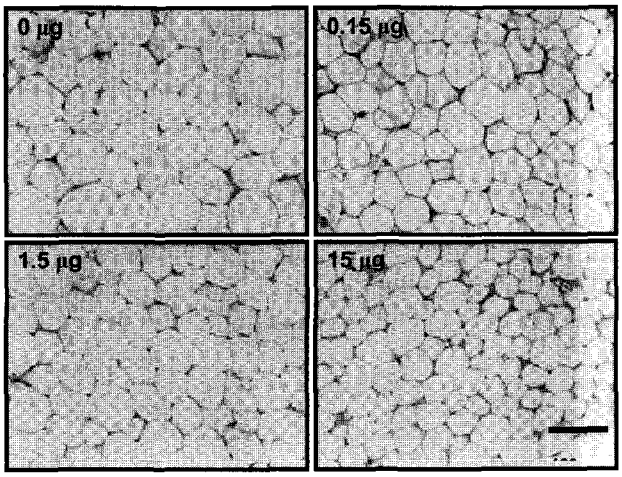

Fig. 2. Effects of 17β-estradiol on morphological changes in adipose tissue of female OVX mice. Mice received intraperitoneal *injections of 17β-estradiol at the indicated doses* for 8 days. Chow diet-fed control mice were administrated corn oil. The parameterial adipose tissues were stained with hematoxylin and eosin (original magnification, ×200).

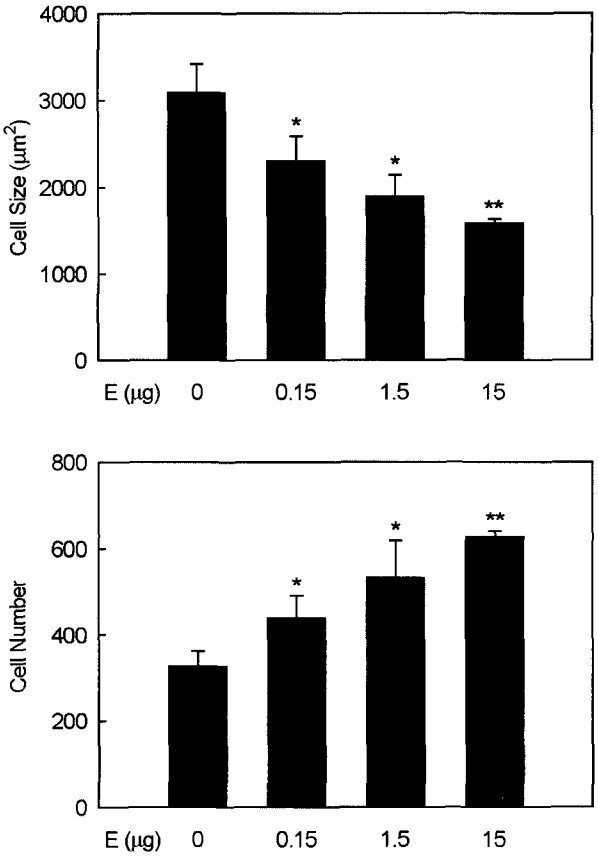

Fig. 3. Effects of 17β-estradiol on number and size of parameterial adipocytes in female OVX mice. Number of adipocytes and their sizes in a fixed area (1,000,000 μm²) were quantified by an image analysis system. Mice received intraperitoneal injections of 17β-estradiol at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. Size and number of adipocytes were measured and all values are expressed as mean ± SD. *, Significantly different *versus* control group ($P<0.05$). **, Significantly different *versus* control group ($P<0.01$).

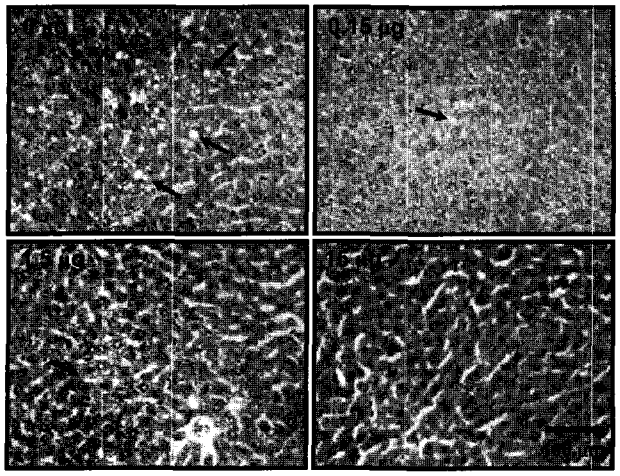

Fig. 4. Effects of 17β-estradiol on morphological changes in hepatic lipid accumulation in female OVX mice. Mice received intraperitoneal injections of 17β-estradiol at the indicated doses for 8 days. Chow diet-fed control mice were administrated corn oil. The livers were stained with hematoxylin and eosin (original magnification ×200). Arrows indicate the fatty changes in hepatocytes.

DISCUSSION

Gonadal sex steroid hormones are known to regulate obesity and lipid metabolism (Mystkwski et al., 2000). Since estrogen is also shown to reduce body weight gain and adipose tissue mass in human and animal models, estrogen may be involved in the regulation of adipose tissue growth. This study was therefore undertaken to determine whether 17β-estradiol modulates adipogenesis in female OVX C57BL/6J mice. Our results demonstrate that 17β-estradiol treatment decreased adipose tissue mass, size of adipocyte, and hepatic lipid accumulation in female OVX mice and these effects were dose-dependent, suggesting that 17β-estradiol treatment regulates adipocyte development and hepatic lipid metabolism.

According to recent reports, OVX animals and postmenopausal women show increased food intake, body weight, and adipose tissue mass (Czaja et al., 1983; Garcia Rodriguez et al., 1990; Geary et al., 2001; Wade 1975; Wing et al., 1991). In addition, estrogen receptor (ER) knockout mice showed significant increases in white adipose tissue with advancing age (Heine et al., 2000). Consistent with our present data, estrogen treatment in postmenopausal women for 1 year, however, decreased intra-abdominal and intrapelvic fat compartments (Mattrasson et al., 2002), as well as improved obesity, adiposity and lipid disorders when OVX rodents are given estrogen (Shearer et al., 2000; Shinoda et al., 2000).

Adipose tissue is composed of adpocytes. The development of fat cells, or adipogenesis, includes morphological changes, cessation of cell growth, expression of many lipogenic enzymes, extensive lipid accumulation, and establishment of sensitivity to most or all of key hormones that impact on this cell type (Rosen and Spiegelman, 2000). With respect to morphological changes and lipid accumulation during adipogenesis, 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 WAT lipids are largely derived from circulating triglycerides, adipocyte size seems to be influenced by serum triglycerides (Bourgeois et al., 1983). In addition, serum lipid levels were reduced by hepatic lipid catabolism (Yoon et al., 2003; Jeong et al., 2004; Jeong et al., 2006). Thus, we suggest a possibility that reduction of hepatic lipid accumulation by 17β-estradiol affects adipocyte size, resulting in decreased the adipose tissue mass.

Estrogen influences energy balance. While energy intake was similar in wild-type and ERα knockout male mice, energy expenditure was reduced in the ERα knockout male mice, indicating that reduction of energy expenditure by estrogen non-action may be related to obesity in these animals (Heine et al., 2000). Estrogen-treated OVX rats increased an uncoupling protein-1 (UCP1) and UCP2 expression of brown adipose tissue as well as UCP2 expression of white adipose tissue compared with OVX controls (Pedersen et al., 2001). Therefore, these reports support our results showing the regulation of adipose tissue mass by estrogen.

The ovarian hormones exert a significant control on cellular lipid homeostasis in metabolically active tissues such as liver and skeletal muscle (Campbell et al., 2001a). In skeletal muscle of OVX rats, treatment with 17β-estradiol upregulates the activity of both carnitine palmitoyltransferase 1 and β-3-hydroxyacyl CoA dehydrogenase, key enzymes in lipid oxidation (Campbell et al., 2001b; Campbell et al., 2003). In addition, exogenous 17β-estradiol increases the hepatic expression of fatty-acid metabolizing enzymes in aromatase-deficient mice (Nemoto et al., 2000; Toda et al., 2001). Although there are conflicting reports that 17β-estradiol regulates the activity of lipid oxidative

enzymes in liver, 17β-estradiol decreases body weight, adipose tissue mass and hepatic lipid accumulation (Gower et al., 2001), suggesting that 17β-estradiol prevents adipocyte development and lipid metabolisms via direct and indirect mechanism in liver.

In conclusion, our results provide morphological evidence that 17β-estradiol regulates adipocyte development and lipid metabolism in a dose-dependent manner. Further experiments will be required to determine how 17β-estradiol affects adipose accretion and obesity.

Acknowledgements

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REFERENCES

Bjorntorp P. The regulation of adipose tissue distribution tissue distribution in humans. Int J Obes. 1996. 20: 291-302.

Bourgeois E, Alexiu A, Lemonnier D. Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice. Br J Nutr. 1983. 49: 17-26.

Bray GA. Risks of obesity. Endocrinol Metab Clin North Am. 2003. 32: 787-804.

Campbell SE, Febbraio MA. Effects of ovarian hormones on exercise metabolism. Curr Opin Clin Nutr Metab Care 2001a. 4: 515-520.

Campbell SE, Febbraio MA. Effects of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. Am J Physiol Endocrinol Metab. 2001b. 281: E803-808.

Campbell SE, Mehan KA, Tunstall RJ, Febbraio MA, Cameron-Smith D. 17β-estradiol upregulates the expression of peroxisome proliferator-activated receptor α and lipid oxidative genes in skeletal muscle. J Mol Endocrinol. 2003. 31: 37-45.

Czaja JA, Butera PC, McCaffrey TA. Independent effects of estradiol on water and food intake. Behav Neurosci. 1983. 97: 210-220.

Garcia Rodriguez LA, Pfaff GM, Schumacher MC, Walker AM, Hoffmeister H. Replacement estrogen use and body weight index. Epidemiology 1990. 1: 219-223.

Geary N, Asarian L. Estradiol increases glucagon's satiating potency in ovariectomized rats. Am J Physiol Regul Integr Comp Physiol. 2001. 281: R1290-R1294.

Gower BA, Nagy TR, Blaylock ML, Wang C, Nyman L. Estradiol may limit lipid oxidation via Cpt 1 expression and hormonal mechanisms. Obes Res. 2002. 10: 167-172.

Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci USA. 2000. 97: 12729-12734.

Jeong S, Kim M, Han M, Lee H, Ahn J, Kim M, Song Y-H, Shin C, Nam K-H, Kim T, Oh GT, Yoon M. Fenofibrate prevents obesity and hypertriglyceridemia in low-density lipoprotein-null mice. Metabolism 2004. 53: 607-613.

Jeong S, Yoon M. Troglitazone lowers serum triglycerides with sexual dimorphism in C57BL/6J mice. J Exp Biomed Sci. 2006. 12: 65-72.

Mattrasson I, Rendell M, Tornquist C, Jeppsson S, Hulthen UL. Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. Horm Metab Res. 2002. 34: 583-588.

Mystkowski P, Schwartz MW. Gonadal steroids and energy homeostasis in the leptin era. Nutrition 2000. 16: 937-946.

Nemoto Y, Toda K, Ono M, Fujikawa-Adachi K, Saibara T, Onishi S, Enzan H, Okeda T, Shizuta Y. Altered expression of fatty acid-metabolizing enzymes in aromatase-deficient mice. J Clin Invest. 2000. 105: 1819-1825.

Ogawa R, Mizuno H, Waranabe A, Migita M, Hyakusoku H, Shimada T. Adipogenic differentiation by adipose-derived stem cells harvested from GFP transgenic mice-including relationship of sex difference. Biochem Biophys Res Commun. 2004. 319: 511-517.

Pedersen SB, Bruun JM, Kristensen K, Richelsen B. Regulation of UCP1, UCP2, and UCP3 mRNA expression in brown adipose tissue, white adipose tissue, and skeletal muscle in rats by estrogen. Biochem Biophys Res Commun. 2001. 288: 191-197.

Rosen ED, Spiegelman BM. Molecular regulation of aidpogenesis. Annu Rev Cell Dev Biol. 2000. 16: 145-171.

Shearer GC, Joles JA, Jones H, Walzem RL, Kaysen GA. Estrogen effects on triglyceride metabolism in analbuminemic rats. Kidney Int. 2000. 57: 2268-2274.

Shinoda M, Latour MG, Lavoie JM. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. Int J Obes Relat Merab Disord. 2000. 26: 335-343.

Tchernof A, Calles-Escandon J, Sites CK, Poehlman ET. Meno-

pause, central body fatness, and insulin resistance: effects of hormone-replacement therapy. Coron Artery Dis. 1998. 9: 503-511.

Toda K, Takeda K, Akira S, Saibara T, Okeda T, Onishi S, Shizuta Y. Alterations in hepatic expression of fatty-acid metabolizing enzymes in ArKO mice and their reversal by the treatment with 17 beta-estradiol or a peroxisome proliferator. J Steroid Biochem Mol Biol. 2001. 79: 11-17.

Villena JA, Viollet B, Andreelli F, Kahn A, Vaulont S, Sul HS. Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit. Diabetes 2004. 53: 2242-2249.

Wade GN. Some effects of ovarian hormone on food intake and body weight in female rats. J Comp Physiol. 1975. 88: 183 -193.

Wade GN, Gray JM. Gonadal effects on food intake and adiposity: A metabolic hypothesis. Physiol Behav. 1979. 22: 583-593.

Wing RR, Matthews KA. Weight gain at the time of menopause. Arch Intern Med. 1991. 151: 97-102.

Yagi K, Kondo D, Okazaki Y, Kano K. A novel preadipocyte cell line established from mouse adult mature adipocytes. Biochem Biophys Res Commun. 2004. 321: 967-974.

Yoon M, Jeong S, Lee H, Han M, Kang J, Kim EY, Kim M, Oh GT. Fenofibrate improves lipid metabolism and obesity in ovariectomized LDL receptor-null mice. Biochem Biophys Res Commun. 2003. 302: 29-34.