

Effects of treadmill exercise training on liver fat accumulation and estrogen receptor alpha expression in intact and ovariectomized rats with or without estrogen replacement treatment

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Abstract To explore the mechanism(s) of exercise training on ovariectomized (OVX)-induced liver lipid disorder, we observed effects of treadmill training on liver fat accumulation and ERalpha expression in intact and ovariectomized rats. Sixty female rats were randomly assigned to six groups: Sham sedentary (S-S), Sham exercised (S-EX), ovariectomized sedentary (O-S), ovariectomized exercised (O-EX), ovariectomized injected subcutaneously with 17beta-estradiol (E_2) (O- E_2), and ovariectomized treated with E_2 and exercise (O- E_2 -EX). Twelve weeks after intervention, OVX resulted in significantly higher body weight gain, intra-abdominal fat mass, serum levels of total cholesterol (TC), and liver triacylglycerol (TAG) concentrations and ERalpha expression than S-S group, while the relative uterus and liver mass, serum levels of E_2 , TAG, and the ratio of high density lipoprotein (HDL) to TC were markedly lower in O-S group. All of these changes were decreased in O-S rats after treatment with E_2 alone with the exception of serum TC and HDL-C levels and liver ERalpha expression. Exercise alone significantly reversed the effect of OVX on serum E_2 , the ratio of HDL-C to TC and the liver and intra-abdominal fat accumulation in OVX rats. The addition of E_2 to exercise induced the same uterus and lipid profile as E_2 alone. Moreover, an additive effect of exercise and E_2 was observed on liver ERalpha expression in Sham or OVX rats. In conclusion, treadmill training alone could prevent liver fat

accumulation in OVX rats and the regulation of exercise on liver ERalpha expression in both OVX and Sham rats needs the presence of physical estrogen levels.

Keywords Exercise · Estrogen replacement · Ovariectomy · Serum and liver lipids · Estrogen receptor

Introduction

For menopausal women, the failure of ovary function easily leads to obesity, especially visceral obesity. As a result, diseases occurrence rate associated with obesity increase and make it essential to prevent postmenopausal obesity that occurs as the hormone levels are reduced (Thurston et al. 2008). At present, there are few effective therapies for postmenopausal obesity except hormone replacement therapy. However, many experiments indicated that hormone replacement therapy has some adverse effects, such as an increased incidence of breast and reproduction system cancer (Chen 2008).

Ovariectomized (OVX) animals have been used as models for obesity from limited estrogen function. It is well known that reduced energy expenditure and increased adiposity and insulin resistance occur as a result of ovariectomy in rodents (Rogers et al. 2009; Saengsirisuwan et al. 2009). In OVX rodents, estrogen replacement and endurance training reduced body weights, visceral fat, and low-density lipoprotein cholesterol (LDL-C) level and corrected the diminished insulin action on skeletal muscle glucose transport (Choi et al. 2005; Gollisch et al. 2009; Saengsirisuwan et al. 2009). Although recent evidence demonstrated the additive benefits of resistance training and restrictive diet on liver fat gain in conditions with metabolic disturbances caused by prolonged estrogen deprivation, the

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effects of endurance exercise alone on liver fat accumulation in condition with ad libitum feeding have not been investigated (Pignon et al. 2009; Corriveau et al. 2008).

Estrogen exerts a variety of important physiological effects, which have been suggested to be mediated via the two known estrogen receptors (ERs), alpha and beta. 17β -estradiol (E_2) binds ERalpha with a higher affinity than ERbeta and promotes higher rates of ERalpha-mediated transcriptional activity at the estrogen response elements (ERE). Liver is one of the well-established target tissues for estrogens. Corroborating data indicate that estrogens influence glucose metabolism through the activation of the ERalpha (Riant et al. 2009) and endurance training is associated with modification of ER transcripts levels in the liver and some of adaptations to endurance training in liver may be mediated by an ER-dependent mechanism (Paquette et al. 2007a). Since receptor levels influence target tissue responsiveness to the hormonal milieu, there has been a great interest in the understanding of how the liver ERalpha, especially in the protein level, is regulated by estrogen and exercise. In light of above-mentioned information, we determined the effects of regular treadmill exercise on serum lipid profiles, liver fat accumulation and ERalpha expression in intact and ovariectomized rats with or without estrogen replacement treatment in the present paper.

Materials and methods

Animals and treatments

Twelve-week-old female Sprague–Dawley rats weighing 235 ± 8 g were supplied by animal department of Peking University and were given regular rat chow and water ad libitum. The housing unit was maintained at $24 \pm 1^\circ\text{C}$ with a light–dark cycle of 12 h. Animal procedures were approved by Capital Institute of Physical Education. Rats were randomly assigned to two groups: Sham operation (SHAM, $n = 20$) or bilateral ovariectomized group according to their body weight ($n = 40$). Both ovaries were excised in OVX group under anesthesia through bilateral skin incision. In Sham group, only fat near ovaries were excised instead of ovaries. After recovery for 7 days, the OVX rats were further divided into the following four groups: OVX sedentary (O-S, $n = 10$), OVX injected with 17β -estradiol (O+E₂, $n = 10$), OVX treated with treadmill exercise training (O+EX, $n = 10$), or OVX treated with treadmill exercise training and 17β -estradiol (O+EX+E₂, $n = 10$). At the same time, the Sham rats were further assigned into the following two groups: Sham sedentary (S-S, $n = 10$) or Sham treated with treadmill exercise training (S+EX, $n = 10$). The O+E₂ and O+E₂+EX rats were subcutaneously injected with 0.1 ml of olive oil/ethanol with 50 μg 17β -estradiol (Sigma Chemical,

St Louis, MO, USA) three times per week for 12 weeks, whereas the other groups were injected subcutaneously with 0.1 ml of olive oil/ethanol. After 2 weeks of accustoming exercise at gradually increasing exercise intensity (10–18 m/min at 0% grade for 15–60 min/days), exercised-trained rats began to run 60 min at a speed of 18 m/min in the morning on the little animal treadmill 5 days per week for 12 weeks. Body weight was measured weekly throughout the experiment.

Tissue collection

At the end of the experiment, the rats were killed about 24 h after the last E_2 treatment and/or the final exercise treatment in order to avoid acute effects. Blood was collected from abdominal artery under anesthesia and allowed to clot for 2 h at room temperature. Serum was separated and stored at -80°C until assayed. Immediately after completion of blood collection, intra-abdominal fat, liver, and uterine were removed and wet mass measured. Some liver tissue specimens were frozen in liquid nitrogen immediately after being weighted and stored at -80°C , and the others were fixed in 4% paraformaldehyde solution overnight and embedded in paraffin.

Serum E_2 and lipids

Serum E_2 was measured by radio immunoassay (RIA). Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TAG) were determined using kits from Zhongsheng Biotechnology Company (Beijing, China).

Immunohistochemistry

Immunohistochemistry was performed according to our previous report (Bu et al. 2006). In brief, tissue sections were deparaffinized, rehydrated, and rinsed with PBS. After that they were microwaved in 10 mM citric acid (pH 6.0) for 15 min at 92 – 98°C for antigen retrieval, then cooled to room temperature. After washing in PBS containing 0.1% BSA, nonspecific binding was blocked in 10% normal goat serum in PBS for 1 h. Then the sections were then incubated with a diluted rabbit polyclonal antibody raised to the full-length recombinant human ER α (H-184; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C in a humidified chamber. The antibody was diluted 1:100 in PBS. After incubation with the primary antibody, the tissues were washed with PBS and were subsequently incubated with biotin-conjugated secondary goat anti-rabbit antibodies (VECTOR, 1:200 dilution in PBS) for 1 h at 37°C . The sections were washed in PBS and incubated with Horseradish Peroxidase Streptavidin for another 1 h at 37°C . After rinsing in PBS, the primary antibody binding was visualized with a

diaminobenzidine (DAB) kit (VECTOR) according to the manufacturer's instructions. Tissues were then washed with water. Counterstaining was done with hematoxylin. To ensure antigenic specificity, parallel experiments were performed using goat preimmune IgG as a negative control.

Immunoblotting

Immunoblotting was also performed according to our previously reported (Bu et al. 2006), but the detection system was changed. In brief, about 20 µg proteins from each sample were loaded onto 10% SDS-PAGE and transferred onto a PVDF membrane (MILLPORE). Membranes were then incubated in 5% nonfat dry milk in TBS for 1 h. Primary antibody for ER (1:400) or beta-actin (SC-1616R, 1:500) was incubated with the membrane overnight at 4°C. The seconded antibody, alkaline phosphatase-conjugated Goat Anti-Rabbit IgG (Vector) was diluted to 1:1,000 and incubated with the membrane for 1 h at room temperature. After the last washing step, NBT-BCIP (Zymed, American) detection was carried out according to the manufacturer's instructions.

Statistical analysis

Data were presented as mean ± SEM. The statistical analysis for immunohistochemistry was performed by Chi-square testing. The statistical analysis for other results was performed using one-way analysis of variance (ANOVA), and significant differences among groups were defined by $P < 0.05$.

Results

Body weight and tissue weights

Initial body weight was not significantly different between groups (Table 1). At the end of the study, the body weight

and body weight gain in the O-S group was significantly increased compared to the S-S group, as expected. There was no difference in body weight and body weight gain between the O-S group and O+EX group. In response to E₂ treatment, O+E₂ and O+EX+E₂ groups showed reduction in final body weight and body weight gain compared with O-S group. In addition, the body weight and body weight gain in the O+EX+E₂ group was significantly increased compared to the O+E₂ group and decreased compared to the O+EX group (Table 1).

Intra-abdominal fat mass was also shown in Table 1. Compared with S-S rats, an increase in intra-abdominal mass was observed in O-S animals, which was suppressed by intervention of estrogen replacement alone or exercise training alone or combined of estrogen replacement and exercise training. However, the combination of estrogen and exercise treatment did not show additive effects.

There was no difference in liver mass between all groups (Table 1). However, O-S rats exhibited decreased relative liver mass compared with S-S control and estrogen replacement increased relative liver mass to a greater degree than S-S control. Exercise was not able to increase relative liver mass in the absence of estrogen replacement (Table 1).

E₂ levels in serum, uterine mass, and relative uterine mass

The concentration of serum E₂ was significantly lower in O-S rats in comparison with S-S rats (Fig. 1a). The OVX rats treated with estrogen replacement alone and combine of exercise and estrogen replacement had significantly higher E₂ levels than rats in O-S group and had almost similar values compared with the S-S group. Although the OVX rats treated with exercise alone had significantly higher E₂ levels than rats in O-S group, still had significantly lower values compared with the S-S group (Fig. 1a).

Table 1 Initial and final body weight, fat, and liver weight in six groups

Parameters	S-S	S-EX	O-S	O-EX	O-E ₂	O-E ₂ -EX
BW (g)						
Initial weight	240.10 ± 12.97	239.00 ± 10.89	240.70 ± 14.70	236.40 ± 20.75	240.30 ± 14.16	241.50 ± 14.89
Final weight	315.98 ± 11.14	318.54 ± 6.31	367.02 ± 14.45**	364.63 ± 20.56	283.90 ± 10.80 ^{##}	310.91 ± 14.42 ^{##}
BW gain	80.98 ± 5.03	80.34 ± 6.13	131.85 ± 10.78**	116.80 ± 10.17	42.54 ± 5.33 ^{##}	62.24 ± 4.76 ^{##}
FW (g)	13.65 ± 1.65	10.91 ± 0.71	20.95 ± 1.09**	10.95 ± 0.78 ^{##}	5.39 ± 0.67 ^{##}	7.54 ± 0.75 ^{##}
FW/kgBW	42.56 ± 4.41	34.34 ± 2.43	54.42 ± 2.67**	32.10 ± 2.85 [#]	19.26 ± 3.83 ^{##}	24.55 ± 2.41 ^{##}
LW(g)	7.92 ± 0.35	7.85 ± 0.33	7.88 ± 0.45	7.19 ± 0.24	8.55 ± 0.23	7.07 ± 0.40
LW/kgBW	25.44 ± 1.58	24.40 ± 0.97	21.38 ± 0.43*	19.93 ± 0.95	26.40 ± 1.08 [#]	24.90 ± 0.44 [#]

Values are mean ± SEM for 8–10 rats per group

BW body weight, FW fat weight, LW liver weight

* $P < 0.05$, ** $P < 0.01$ versus S-S group, # $P < 0.05$, ^{##} $P < 0.01$ versus O-S group

Fig. 1 Serum levels of estradiol (a), uterus morphology (b), uterus mass (c), and relative uterus mass (d) in six groups. S S-S group, S-E S-EX group, O O-S group, O-E O-EX group, O-E₂ O-E₂ group, O-E-E₂ O-EX-E₂ group. Values are mean \pm SEM for 8–10 rats per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus S-S group; # $P < 0.05$, ## $P < 0.01$ versus O-S group

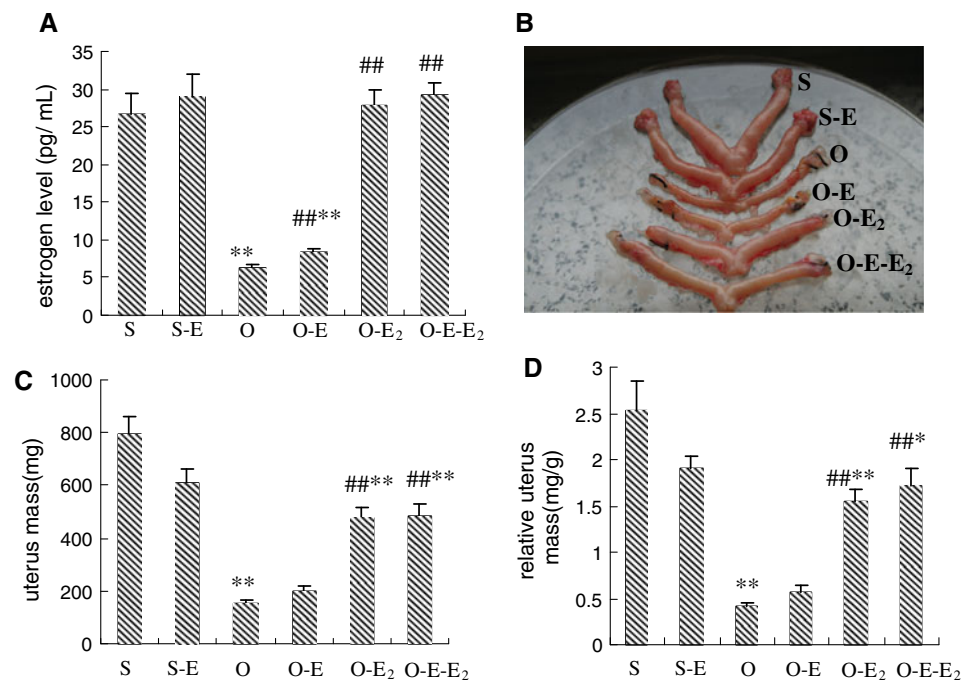


Table 2 Serum TC, TAG, HDL-C, and HDL-C/TC in six groups after 14-week experimental period

Groups	TC (mmol/L)	TAG (mmol/L)	HDL-C (mmol/L)	HDL-C/TC
S-S	1.5924 \pm 0.1865	0.5947 \pm .0543	0.8009 \pm 0.1253	0.4942 \pm 0.0210
S-EX	1.4392 \pm 0.1239	0.3071 \pm 0.0124**	0.7189 \pm 0.0532	0.5030 \pm 0.0153
O-S	2.3391 \pm 0.1729*	0.3644 \pm 0.0206**	0.9209 \pm 0.0550	0.3927 \pm 0.0189**
O-EX	1.9554 \pm 0.02926	0.3824 \pm 0.0185	0.8965 \pm 0.0753	0.4564 \pm 0.0279#
O-E ₂	2.3021 \pm 0.3416	0.4636 \pm 0.0353#	1.0126 \pm 0.0992	0.4636 \pm 0.0226#
O-EX-E ₂	2.2028 \pm 0.3064	0.4477 \pm 0.0197##	0.9445 \pm 0.00904	0.4531 \pm 0.0194#

Values are mean \pm SEM for 8–10 rats per group

TC total cholesterol, TAG triacylglycerol, HDL-C high density lipoprotein

* $P < 0.05$, ** $P < 0.01$ versus S-S group; # $P < 0.05$, ## $P < 0.01$ versus O-S group

As shown in Fig. 1b, the uterus of OVX rats became thin compared with Sham rats and they returned thick after treatment with estrogen replacement alone or combination of exercise and estrogen replacement.

Ovariectomy caused a significant reduction in the uterus mass (Fig. 1c) and relative uterus mass (Fig. 1d) compared with S-S group. The uterus mass and relative uterus mass of O+E₂ and O+EX+E₂ groups were significantly higher than O-S and O+EX groups. However, there was no difference between O+EX group and O-S group (Fig. 1c, d).

Serum lipids

Serum levels of TC, TAG, HDL-C, and HDL-C to TC ratio were demonstrated in Table 2. Compared with Sham rats, ovariectomy led to a significant increase in TC and a significant decrease in TAG and HDL-C to TC ratio. Exercise

training alone significantly reduced TG levels in Sham rats but had no effect on OVX rats. In contrast, exercise training alone significantly increased HDL-C to TC ratio in OVX rats but had no effect on Sham rats. Estrogen replacement alone produced an increase in TG levels without any significant change in TC, HDL-C, and HDL-C to TC ratio compared with the O-S group. Combined intervention brought about the same lipid profile as estrogen replacement alone.

Liver TAG

As shown in Fig. 2, the content of liver TAG was significantly reduced after exercise treatment in both Sham and OVX group. OVX rats exhibited increased liver TAG compared with S-S control and this increase could be reversed by either exercise alone or estrogen replacement alone or combination intervention (Fig. 2).

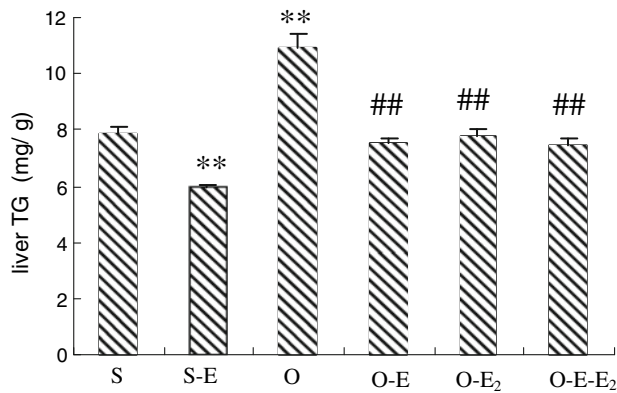


Fig. 2 Liver triacylglycerol concentration (TG) concentrations in six groups. *S* S-S group; *S-E* S-EX group; *O* O-S group; *O-E* O-EX group; *O-E₂* O-E₂ group; *O-E-E₂* O-EX-E₂ group. Values are mean \pm SEM for 8–10 rats per group. * $P < 0.05$, ** $P < 0.01$ versus SHAM group; # $P < 0.05$, ## $P < 0.01$ versus OVX group

Liver ER α protein level

Immunostaining with ER α antibody showed that ER α was mainly expressed in the nuclei of liver cells in our studied groups (Fig. 3).

The number of ER α -positive spots and the OD of ER α protein in liver was respectively shown in Table 3 and Figs. 4 and 5. The number of ER α -positive spots and the OD of ER α protein in liver of intact rats increased significantly after endurance training (Table 3; Fig. 5) or OVX treatment (Table 3; Figs. 4, 5). In addition, there was significant increase in the number of ER α -positive spots

and the OD of ER α protein when combining exercise with E₂ compared with O-S controls (Table 3; Fig. 5). In contrast, the expression of ER α protein was unaffected when OVX animals received the treatment with exercise or E₂ alone (Table 3; Figs. 4, 5).

Discussion

Estrogen deficiency easily leads to overweight or obesity after menopause and physical exercise is one of the important modulators of this body weight gain (Green et al. 2004; Stefanick et al. 1998). The major finding of the present investigation was to emphasize the independent role of regular treadmill exercise in the regulation of liver fat accumulation and its additive role with estrogen in the stimulation of liver ER α expression in OVX rats. In the present study, we found that OVX rats exhibited increased body weight gain, visceral fat content, and liver TAG as well as serum TC. These obesity characteristics were partly corrected by either treadmill exercise alone or E₂ replacement alone, whereas the beneficial interactive effects of exercise and E₂ on these defects were not apparent. To our knowledge this is the first study to demonstrate that a moderate treadmill exercise induced increase in liver ER α expression was associated with the content of serum E₂.

Data from present study indicated that OVX resulted in a significant reduction in circulating serum E₂ levels and uterus weight compared with that shown in the Sham rats and these reductions were reversed by supplement of E₂. It

Fig. 3 Immunohistochemistry detection of ER α localization in liver sections of all groups. Strong ER α staining is detected in nuclei of hepatocytes. No ER α staining is detected on the consecutive sections with Sham rats when nonimmune IgG is utilized as primary antibody (g). Bar 50 μ m. **a** S-S group; **b** S-EX group; **c** O-S group; **d** O-EX group; **e** O-E₂ group; **f** O-EX-E₂ group; **g** negative group

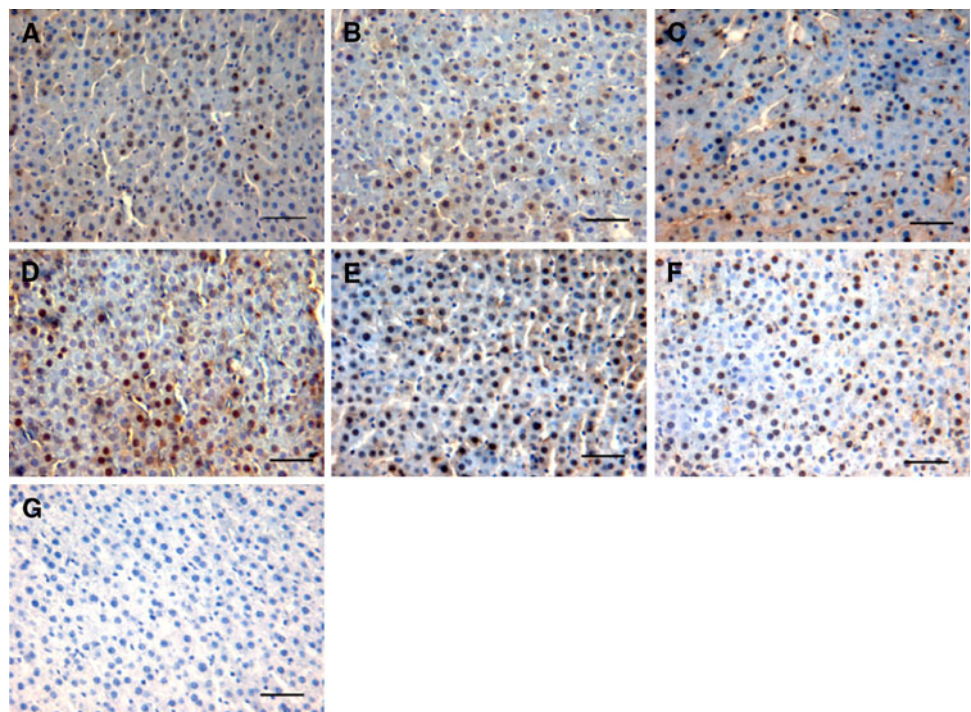


Table 3 The positive cell number of ER α per 500 cells in all groups

Parameters	S-S	S-EX	O-S	O-EX	O-E ₂	O-EX-E ₂
Positive cell number/500 cell	115	300**	201**	224	202	326 ^{##&&}

** $P < 0.01$ versus S-S, ^{##} $P < 0.01$ versus O-S, ^{&&} $P < 0.01$ versus O-EX

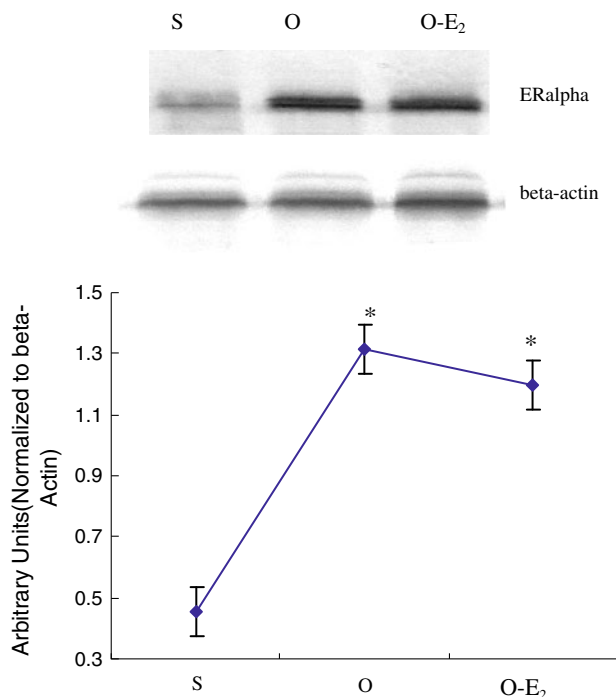


Fig. 4 Western blot analysis showing ER α (66 kDa) and beta-actin (42 kDa) in liver tissue sampled from S-S(S), O-S(O), and O-E₂ (O-E₂) rats. The mean \pm SEM has been calculated in four experiments. * $P < 0.05$ versus S-S

is suggested that both the OVX surgery and estrogen replacement were successful. Although natural menopause or OVX is associated with loss of ovarian estrogen production, other organs such as fat, muscle, vessels and brain can also produce some content of estrogens after menopaual or OVX. This explains why the OVX rats still had 20% of normal serum E₂ at the end of the experiment in this study. Moreover, an interesting observation was that exercise increased serum E₂ level in OVX rats but not in Sham rats. Further study will be needed to investigate the mechanism by which exercise exerted influence on serum E₂ under the loss of ovarian function.

It is well documented that OVX results in a significant weight gain and an alteration in body composition. This is most likely produced by an increase in food intake and a decrease in energy expenditure (Saengsirisuwan et al. 2009; Rogers et al. 2009). In the present study, the first observation was that treadmill exercise alone did not reduce OVX induced greater weight gain. But the estrogen replacement reduced the

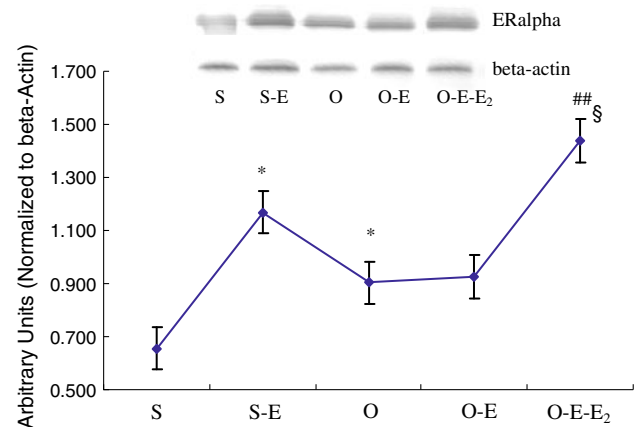


Fig. 5 Western blot analysis showing ER α (66 kDa) and beta-actin (42 kDa) in liver tissue sampled from S-S(S), S-EX (S-E), O-S(O), O-EX (O-E), and O-EX-E₂ (O-E-E₂) rats. The mean \pm SEM has been calculated in four experiments. * $P < 0.05$ versus S-S; ^{##} $P < 0.01$ versus O-S; _{\$} $P < 0.05$ versus O-E

weight gain below the values observed in the S-S group, whereas the combined treatment with exercise reduced the weight gain to the midway between O-S and S-S rats. These findings were consistent with the previous reports that there were no significant main effects of training on either body weight or food intake between exercise group and sedentary group in OVX rats (Latour et al. 2001). Moreover, similar results were reported by Velthuis et al. (2009) who found that exercise program affected body composition but not weight in postmenopausal women. This did not mean body composition was not changed by training. In fact, the exercise group experienced a significant greater loss in intra-abdominal fat, both absolute and relative compared with the O-S group.

Obesity easily leads to disorder of serum lipids (Durstine and Thomson 2001; Lee et al. 2007). At present, the reports on the effects of exercise training on serum lipids disorder in OVX rats are not consistent. Saengsirisuwan et al. (2009) observed that exercise training alone significantly reduced TC without any significant change in HDL-C and the ratio of HDL-C to TC. However, Mohanka et al. (2006) reported that exercise training had no effects on serum lipids. In the present study, regular treadmill exercise effectively increased the ratio of HDL-C to TC in OVX rats but had not effect on the levels of TC and HDL-C. These results were partly contrary to the above report by Saengsirisuwan et al. (2009). We supposed that the major differences between the two studies might be the duration of sex hormone deficiency and the exercise intensity. In addition, the effect of OVX on serum TAG levels is also controversial. Some studies have reported a reduction in serum TAG levels after OVX (Barsalani et al. 2008; Paquette et al. 2007b), while others have not observed any changes (Bitto et al. 2009; Sanchez-Mateos et al. 2007; Meli et al. 2004). These contradictory results could be due to factors such as the diet, the subject's metabolic conditions and the

duration of the study. In the present study, comparisons between OVX and rats without estrogen deficiency (S-S, O-EX-E₂, and O-E₂) revealed that serum TAG levels in S-S, O-EX-E₂, and O-E₂ rats were higher than levels measured in O-S rats. The results were similar to those reported by Paquette et al. (2007b) who found that plasma TAG levels in Sham and OVX-E₂ rats were higher than levels measured in OVX rats. In addition, 12 weeks of treadmill exercise training did not affect serum TAG levels in O-S and O-E₂ rats, but did lead to a decrease in S-S rats. The results suggested that there might be other factors in addition to estrogen for the decreased TAG levels found in trained animals.

Liver is the major organ for lipids synthesis and metabolism. The change in serum lipids is directly decided by lipid synthesis and metabolism. Significant accumulation of liver TAG has been recently reported in female Sprague–Dawley rats 3–13 weeks after ovariectomy (Paquette et al. 2007b). Corriveau et al. (2008) reported that the addition of resistance training to the restrictive diet treatment synergistically resulted in an important decrease in liver TAG accumulation induced by OVX. They concluded that the effects of resistance training on liver lipid infiltration were independent of the restrictive diet regimen since the restrictive diet had no effects on liver lipid infiltration. This finding is supported by results of the present study showing that treadmill training alone prevented the OVX-induced liver lipid infiltration. In addition, liver fat accumulation was also effectively corrected by estrogen replacement alone in the present study. Since the liver lipid disorder induced by OVX could be effectively corrected by either exercise treatment alone or E₂ treatment alone, it is reasonable in the present study that exercise training could not additively modulate liver lipid infiltration in OVX rats that also received estrogen replacement, suggesting that it is not necessary to use endurance training treatment and estrogen treatment simultaneously only in order to correct defects in liver lipid infiltration caused by estrogen deficiency to the level of Sham rats.

Hormone receptors are generally negative-feedback regulated by the concentrations of their own ligands and this also seems to be the case with estrogen receptors (ER) (Stavreus-Evers et al. 1997). Estrogens exert their biological roles mainly via binding to its tissue receptors. ER content in a cell is regulated by many factors and is decisive for estrogen action. There are two ERs. One is ERalpha, the other is ERbeta. In liver, both ERalpha and ERbeta gene were found, but ERalpha is the main receptor for mediating the signal pathway of E₂. It has been proposed that endogenous E₂ may be repressive on hepatic ER gene expression of intact rats, as an elevation of ERalpha mRNA level is observed after OVX (Paquette et al. 2007a). Accordingly, we found an increase in hepatic ERalpha protein levels after OVX. This indicated that both ERalpha

gene and protein expression were largely modified by the OVX and the increase of ERalpha may constitute a compensation for ovary function in the absence of ovaries. In addition, E₂ replacement did not lower ERalpha to the level of Sham in our present study. This suggests that the increase of ERalpha protein expression induced by OVX might not be only induced by the decrease of serum E₂ level and other factors might be also involved in this process. In fact, previous studies have reported that hepatic ERalpha content is virtually under the control of several factors including pituitary and gonad hormones and the action of E₂ in liver depends on the presence of the pituitary hormones (Freyschuss et al. 1994; Norstedt et al. 1981).

Endurance training has been reported to regulate ERalpha expression in several tissues such as liver, heart and skeletal muscle (Paquette et al. 2007a; Lemoine et al. 2002). Paquette et al. (2007a) has put forward the conclusion that endurance training has different effects on ER transcripts levels in the liver according to the presence or the absence of ovaries. Data from the present study indicated that the training adaptations of ERalpha protein content in the liver of OVX animals were different from the training response observed in Sham rats, but our results were not completely consistent with the results of Paquette et al. (2007a). In other words, our results that exercise training increased ERalpha protein content in the livers of the Sham rats were consistent with the results of Paquette et al. (2007a). However, our results of the effect of endurance training on ERalpha protein expression in livers of OVX rats were different from the results of Paquette et al. (2007a). As for the reason, we thought this might be attributed to the intensity and duration of training (one major difference between the two studies). This interpretation is of interest especially that ERalpha protein levels in livers of OVX rats relied on the intensity and duration of training. It is suggested that the ERalpha protein expression in the liver of OVX rats is more sensitive to exercise stimulus than the ERalpha protein expression in the liver of Sham rats and that an ER-mediated mechanism could contribute to the training adaptations of liver. Peng et al. (1997) have reported that bone in OVX rats is more sensitive to exercise than in Sham-operated rats. Our data suggested that this concept could be extended to liver.

Taking into account the fact that the presence or absence of ovaries influenced the response of ERalpha expression to the stimulus of exercise, we also observed the combined effects of exercise and E₂ in the present paper. The results indicated that the expression of ERalpha protein in livers of OVX rats was significantly increased after combined treatment with exercise and E₂. To our knowledge, our study is the first to report that the modulation of treadmill training on ERalpha protein levels in the liver of female

rats is associated with the level of E₂ and some of the adaptations to endurance training in liver may be mediated by an ER α -dependent mechanism.

Conclusions

In brief, our present paper indicated that treadmill exercise training alone could prevent liver fat accumulation in OVX rats and the regulation of exercise training on liver ER α expression needs the presence of physical estrogen levels in both Sham (normal E₂ levels) and OVX (injected with exogenous E₂) rats.

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Conflict of interest statement None.

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